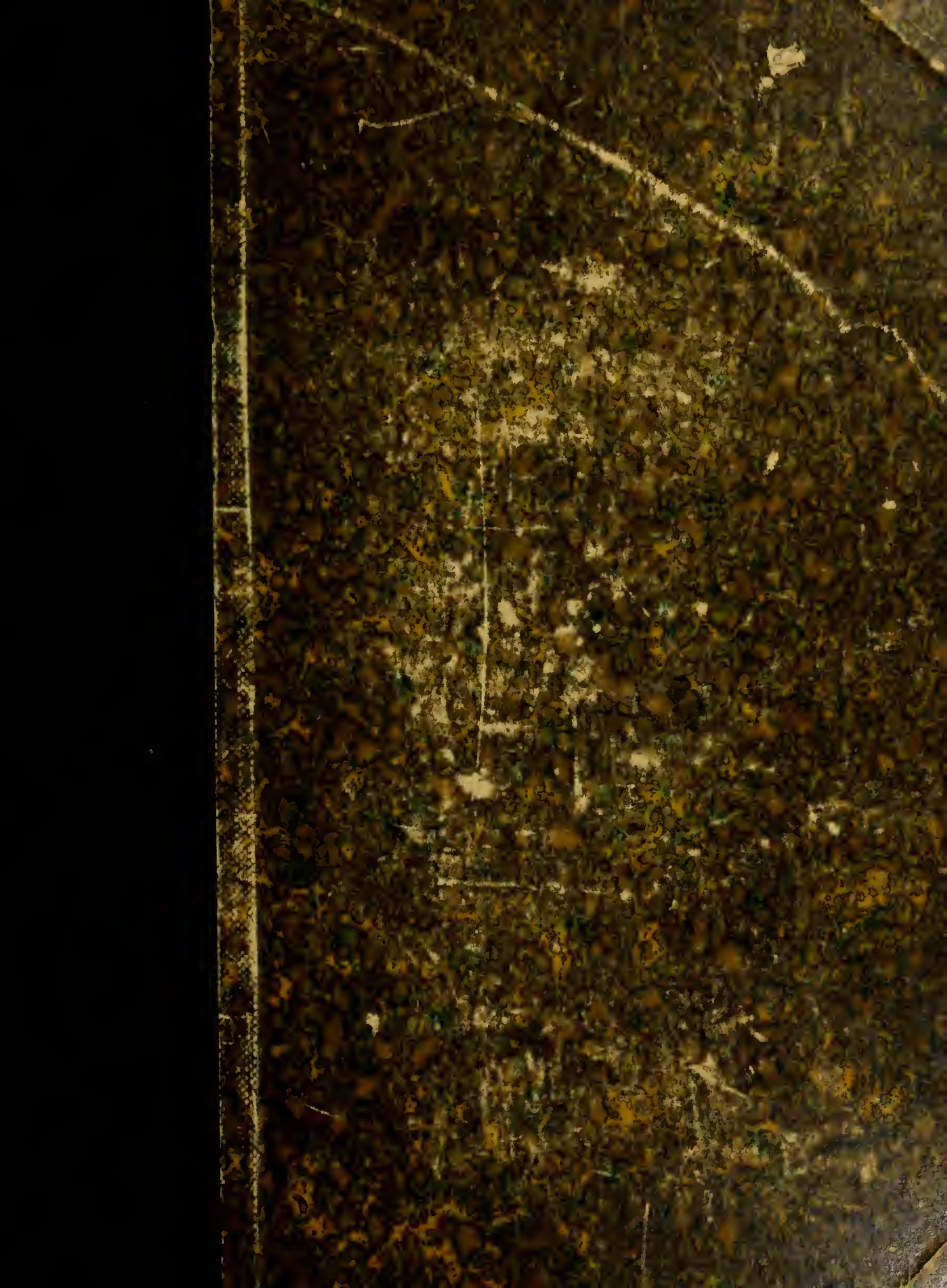

BLOOD-PLATES, COAGULATION, AND REGENERATION OF BLOOD

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED Blood-plates, Coagulation, and Regeneration of Blood

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

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The work done on blood-plates may be divided into two periods. The first period includes the results obtained before 1878, when, owing to primitive methods and inaccurate descriptions, the work published leaves us in doubt as to whether the observers were investigating the real plates, precipitates resulting from the chemical reagents used, or detritus. The second period begins with Hayem's paper "Sur la nature et la signification des petits globules rouges du sang," published in Comptes Rendus de l'Academie des Sciences, Paris, Vol. 84, 1877, and takes in all the work done since. This period is characterized by improved methods of observation, and by careful descriptions which serve to distinguish the plates from foreign bodies, or from granules in the blood. Only the second period needs to be studied in detail; a brief review is sufficient for the first.

In 1842 Donné[1] described small white particles not more than .003 m.m. in diameter, which had a tendency to stick together, and he gave them the name of globulins. These are undoubtedly identical with blood-plates. Before Donné, Müller[2], Mandl, Henle, Wharton Jones, Gerber, Arnold, Andral, and Simon, gave descriptions of colorless bodies, which may have been plates.

Zimmerman[3] (p. 227) in studying his "Elementarkörperchen" (plates), showed for the first time the microchemical reactions of the blood-plates. He believes they originate from the lymph, and claims to have seen them in frog's blood, and in defibrinated blood; which fairly raises the question whether he really distinguished the plates.

Beale,[3] (1864. (p. 42) rather indefinitely mentions three structures occurring in blood which possess some of the characteristics of blood-plates. Max Schultze, 1865. (p. 36-37) describes the granular masses formed by the plates, as well as individual plates. He did some work on the reactions with water, acetic acid, etc. Riess[5] in 1872, (p. 240) examined blood as quickly as possible after it was drawn and found "Kügelchen", which are probably plates and granules.

In describing the arrangement of the fibrin threads in relation to the granules, Ranvier[6] (1873. (p. 215) had reference to plate masses which he considered identical with fibrin. Vulpian[7] (1873. (p. 94) demonstrated the presence of plates in normal blood. Nedsvetzski,[8] (p. 147-150) in the same year, discovered that his "Blutkörnchen" or "Haemococci" had the same relation to the fibrin threads formed during coagulation, as had the fibrin-masses of Ranvier.

Osler[9] in 1874, saw plates in the blood-vessels in the connective tissue of the back of a young rat. Schmidt[10] and Semmer[11] the same year, investigating the granular masses mentioned by Schultze, claimed they were not derived from blood-plates. Boettscher[12] 1877, (p. 398) figures structures which he regards as escaped nuclei of the red corpuscles, but which were probably plates. Norris,[13] (1879, p. 163) claims that he was the original discoverer of the plates, but what he designated as plates were merely shadows of the red corpuscles. This brings us to the first work of Hayem.

The origin of the blood-plates has never been even approximately settled. Several theories have been advanced by different

authors which are as follows:

1. They are derived from the stromata of the red corpuscles.

2. They are derived from the nuclei of the erythrocytes.

3. They are derived from the "Innenkörper."

4. They originate from the protoplasm of the leucocytes.

5. They originate from the leucocyte nuclei.

6. They are fibrin particles. This view was first advanced by Ranvier, in 1873, and has not received any support in recent years.

7. They are precipitated globulins.

8. They exist in the blood, as independent elements.

Of the foregoing theories only three receive sufficient support, at present, to entitle them to detailed consideration. These are: the theories of their origin from the red corpuscles, the theories of their origin from the white corpuscles, and the theory that they are independent elements.

In support of the first two views we have Fusari[14] (p. 246), who found from microchemical tests that the plates resemble the erythrocytes in their action toward eosin, and the protoplasm of the leucocytes in their resistance to the action of water, acetic acid, etc., and in staining with aniline-dyes, i.e. methyl-violet, gentian-violet, and methylene blue.

Mosso[15] is of the opinion that plates are nuclei of red corpuscles, and that these erythrocytes burst setting free their nuclei, which are described as plates. Klebs[16] (p. 134) found in paraffine sections of thrombi, formed in the vein of the liver, that the eosinophile substance came out of the red corpuscles,

and he identified this substance with blood-plates, since they both stain with eosin. Klebs speaks of a degenerative change in which globulin loosened from the stroma is extruded, possibly by the elastic membrane of the erythrocyte. He calls this, the "Globulin or Plättchen degeneration" of the red-corpuscle, and claims it is of frequent occurrence.

Welti[17] (p. 548) found that in animals suffering from scalds, the erythrocytes broke down, giving rise to plates, which in turn formed emboli causing death. He said the number of plates in these cases was usually considerably increased, but he did not use any method of numeration. According to his view, the majority of the plates arise from the red corpuscles, although some may come from the leucocytes. Salvioli[18] (p. 365) repeated and modified Welti's method of studying the blood of animals suffering from scalds. He agrees with Welti that the cause of death was plate-emboli, but he denies that the plates are increased after scalds. On the contrary, his counts show that the plates steadily diminished in number, and his explanation for this was, that plates adhered to the scalded blood-vessels, and also formed emboli, which decreased their number.

Bremer[19], (p. 338) working on smear preparations dried at 120° C. and stained with a watery solution of eosin and methylene-blue, found among the plate masses, broken down red corpuscles in different stages of disintegration. The fragments are often found connected with the plates by a thread of fibrin. He concluded that, since these disintegration-forms are found only in the blood of certain individuals, and are totally lacking in the

blood of most healthy persons, they were not artificial results of the methods used. He found the blue-stained plates coming out from the opened red corpuscles in chains or masses which seemed to him convincing evidence that although the plates exist in normal circulating blood, they are never independent elements.

WLASSOW'S WORK ON THE ORIGIN OF THE PLATES

Wlassow[20] (pp. 559-566) recommends the following methods for showing the origin of the plates. He makes a mixture of paraffine-oil and vaseline so that the oil just ceases to be fluid. He smears a thin layer of this on both the slide and the cover glass. After sticking his finger he places a drop of the grease over the puncture, and squeezes out a small drop of blood which he examines between the greased slide and cover. He says there are no plates and no distorted degeneration forms of the red corpuscles from which the plates, according to his view, are derived. If the blood comes in contact with any ungreased portions of the glass, degenerated red corpuscles and blood-plates are found in these areas. He says he was unable to find any fluid which would fix the degenerating red corpuscles in the act of giving off blood-plates. For a study of the plates Wlassow recommends three fluids. First:

1% Na Cl Sol. ---------- 6 cc

5% $K_2 Cr_2 O_7$ ---------- 4 to 6 drops.

The edges of the cover-glass are smeared with vaseline leaving the centre clear; the blood-drop is squeezed into the fluid on the finger, then it is placed on the slide and left long enough for the plates to rise; the cover-glass with its thick

vaseline ring is put over it. In these preparations, Wlassow finds the plates floating free, and red corpuscles that appear to have broken into two parts which are still connected. One of these pieces is larger, and contains haemoglobin; both appear as star-shaped lumpy discs. Many of the separated plates contain haemoglobin, and vary greatly in form and size, some being star-shaped, branched and tailed. These processes and tails best illustrate the method of origin of the blood-plates: they are formed by the separation of the disintegration products from each other. The number of the blood-plates in comparison with the degenerated erythrocytes is not greater in this fluid, than in Bizzozero's. The other red corpuscles show no special alteration. The microcytes are lacking. This last statement of Wlassow, taken in connection with the assertion that some of the plates contain haemoglobin, makes it appear highly probable to us, that he confused microcytes with plates. This experiment, according to Wlassow, proves that the red corpuscles in 1% Na Cl Solution, break up into two blood-plates, which are made up of protoplasmic as well as nucleo-albumin constituents. The latter forms the humps and spikes of the blood-plates which give rise to the star and "Steck-apfel" forms. The tails and processes consist partly of nucleo-albumin, and partly of protoplasmic constituents. The same forms of degenerative erythrocytes and blood-plates are seen in pure unmixed blood. The second solution differs from the first, only in the presence of about .02 % of osmic acid. The small number of plates is striking, some being colored with haemoglobin, and slightly concave when seen from the surface. The smaller plates

which are free from haemoglobin, and which have no central concavity, represent colorless lenticular blood-plates. This is the usual form seen in 1% osmic acid. Besides these blood-plates, Wlassow sees the two parts of the broken-down red corpuscles still connected by a nucleo-albumin thread. In this fluid as well as in 1% osmic acid solution, the blood-plates are apparently formed at the expense of the red corpuscles, but the number of erythrocytes which break down is less in the first fluid described. The mulberry forms are almost a normal occurrence in osmic acid solutions. The third solution consists of:

Concentrated Solution $Hg\ Cl_2$	2 drops
3% Na Cl Solution	4 c.c.
5% $K_2\ Cr_2\ O_7$ Solution	1 drop

This fluid gives constant results. Wlassow says that the number of blood-plates is small; some of them appearing as discs from 1/3 to 1/2 the size of the red corpuscles, concave, and colored with haemoglobin, while others are smaller, colorless, and biconvex. It is extremely interesting to note that many red corpuscles contain special biconcave or biconvex highly refractive bodies. The former are granular in the centre but homogeneous at the periphery. At the body temperature these granules move toward the periphery of the cell, and escape as independent elements which cannot be distinguished from small homogeneous blood-plates. It is worthy of note that the cells are not altered in form, and Wlassow believes that they are part of the nucleo-albumin constituents of of erythrocytes. At 65° C. the nucleo-albumin of all the red corpuscles passes out in the form of circular concave discs about

1/3 the size of the erythrocytes and granular at the centre with a homogeneous edge. It is characteristic that the same changes are noticed in the large blood-plates, i.e. the extrusion of the previously split albumin part, which the protoplasmic residue of the blood-plates is transformed into spherical granular microcytes just as in the red corpuscles. Hirschfeld[21] (p. 208) corroborates Wlassow's observations that plate-like bodies are found issuing from the red corpuscles when blood is treated with "five fold" diluted corrosive sublimate solution, but he considers these identical with the "Innenkörper" (to be described later- see page .10.) but not identical with plates. Scherer[22] after repeating Wlassow's experiments says that the fluid which Wlassow used, when warmed to 65° C., produces in a small number of red corpuscles a zooid formation, and from most of the red corpuscles, drop-like particles are given off which, although they faintly resemble the plates, yet are clearly distinguished from them by their form and haemoglobin content. Scherer thinks that Wlassow's plate forms are partly zooids and partly broken off protoplasmic constituents of the erythrocytes, which contain haemoglobin. Sacerdotti[23] shows that Wlassow's plate-buds disappear when treated with dilute acetic acid, which seems conclusive proof that all of Wlassow's plates were not genuine since real plates are not dissolved by the acetic acid.

Gibson[24] (p. 200) describes colorless microcytes which he believes to be the nuclei of red corpuscles and of leucocytes. The colorless marrow-cells develop into nucleated red corpuscles;

the nuclei fragment or become plates, colorless microcytes, which are according to Gibson, the same as Hayem's haematoblasts. They are the agents causing coagulation.

ARNOLD'S WORK

Arnold[25] (p. 1) suggests a striking theory concerning the origin of the blood-plates from the red corpuscles. Using a 10% K I Solution, he shows, in the hanging drop, red corpuscles which have granular threads and processes that finally break loose. The free bodies thus formed agree optically and in their staining reactions, with the plates, as can be shown in dry preparations or celloidin sections. These and other reasons support his theory that the plates are "Abschnürungs -oder Ausscheidungs-produckte" of developed red corpuscles, and are against the view that plates are leucocyte nuclei. In the mesenteric circulation, and in the mesenteric vessels, after death, as well as in elder-pith sections he has seen the above mentioned formation of plates. These plate buds of the red corpuscles in fresh and dry preparations from 10% K I solution, show that they are made up of a finely granular protoplasmic substance in which dark granules and masses of granules, are seen. His method is to dry and then stain in aniline-gentian-violet or in methylene-blue and eosin. In such preparations many of the red corpuscles show a finely granulated light centre while the periphery stains a blush violet. Most of the granules are uncolored although some show a violet-reddish or blue tint. Sometimes thread-like connections are seen between the granules or there is a reticular structure throughout the whole "Innenkörper." In using Weigert's fibrin stain, blue dots

appear not only in the centre but also at the periphery. Some lie outside the red corpuscles with which they are connected by blue threads. Furthermore many red corpuscles are decolorized completely by Gram's method, while others retain the stain in the centre. Osmic acid preparations stained in methylene-blue-eosin show many red corpuscles with a blue speck in the centre, although most of them are more uniformly stained red. With iron-haematoxylin and eosin, the differences are even more striking. Darker spots or discs at the centre or periphery indicate the presence of nuclear matter.

Nuclear Matter in Red Corpuscles.

We have not worked on nuclear matter in the ordinary non-nucleated red blood corpuscle but numerous observers have demonstrated its presence, and since we believe the plates contain nuclear matter, it seems to us important to discuss the work done along this line. Löwit[26] found in red corpuscles from the vena-cava and the right side of the heart in rabbits, nucleus-like structures which he called "Innenkörper." These bodies are sometimes in the centre, but more often near the periphery; frequently only a few granules indicate their presence. These granules are sometimes connected by threads, so that a similarity to the nuclear arrangement of chromatin is shown. Löwit felt justified in considering these "Innenkörper" as the remains of nuclei.

Foa[27] observed granules, usually arranged in the centre of the red corpuscle but often in the periphery, that stained with methylene-blue. He assumes that the centre of the corpuscle is the place where the embryonic nucleus had lain, and to which

a granule-bearing network of threads was attached. Howell[28] also holds this last view, and ascribes the biconcavity of the red corpuscles to the loss of the nucleus. Maraglino and Castellino[29] go a step further, and describe two substances in the "Innenkörper."

Lavdowsky,[30] after a thorough study of the subject, decides that the greater part of the adult mammalian red corpuscles contain the remains of a nuclear substance which he calls "nuclecid." These nucleoids occur as small bodies which stain deeply, and are usually centrally located, and they unite with one another to form peculiar figures which he calls "chemotropic." He also finds in the "Innenkörper," radiating lines which give a fan-like structure to the stroma. Wlassow[31] speaks of a nucleo-albumin constituent of the erythrocytes, which passes out of the corpuscle to form plates.

Maximow[32] says that all red corpuscles, without exception, appear to have a nucleus-like structure in the centre, which he, like Löwit, designates as "nucleoid." These nucleoids, with Maximow's stain (see p. 49.) take varying shades of violet, depending on the amount of nuclear matter present. They are not the same size in all corpuscles, the largest being usually about two-thirds the diameter of the erythrocytes. These nucleoids are often homogeneous.

Franz Müller[33] says that plates usually arise by budding or fragmentation of the red corpuscles, although they may come from the leucocytes as well. Determann[34] attempted to avoid the physical and chemical disturbing factors which are objected to in Arnold's

work.

He heated fine glass tubes, drew out the ends and while hot fused them shut, after using antiseptic precautions in drawing the blood, one of the drawn out ends of the tube was cut beneath the surface of the drop; the blood rushed into the partial vacuum and the end was again fused shut. These tubes were kept in an incubator at body temperature, and Determann says: "so könnte ich nach 24, 48, 72 Stunden, nach Tagen und Wochen sehen was aus dem Blut geworden war." He also used the elder-pith method, and ligated pieces of carotid, cutting them out after 1, 2, and 10 days. The blood was fluid in all three cases. Many "Steckapfel" and mulberry forms were present, with but few free-floating plates.

He tends to agree with Arnold that the plates bud off from the red corpuscles, but he claims that the buds are often free from haemoglobin. These buds stain exactly like the ordinary blood-plates, and also like the border zone of the leucocytes with methylene blue, methyl violet, fuchsin, etc. Determann worked on healthy and on pathological cases, and he evidently considered Arnold's work the most conclusive of any then published (1898). Hirschfeld[35], repeating Determann's experiments of enclosing blood in sterile tubes for some time, found the budding processes described by Determann, but he claims that he saw no true plates.

Maximow[36], after working on dry preparations stained with methylene-blue and eosin, believes that plates arise from nuclear material which is extended from the red corpuscles. His work is especially interesting on account of his thorough study of the nuclear matter in the erythrocytes. He discusses the probability

of dealing with artefacts, and describes many appearances which are artificial. The great variety of appearances which he claims are not artificial, as well as his method of using dry preparations, give room for serious criticism.

Hirschfeld[37] agrees with nearly all of Maximow's conclusions, but he does not regard the plates as remnants of nuclear matter. He says the formation of plates in red corpuscles, and their extrusion, is especially easy of demonstration in anaemic blood. Several plates may be found in one red corpuscle, and the corpuscle may extrude one of these, and retain the others. In addition to the plates; he finds platelike structures in the erythrocytes, which are sharply distinguished from the true plates by their stain with haematoxylin and eosin, or methylene-blue-eosin. The true plates stain blue, and the plate-like bodies red. Only a few red corpuscles show the formation of blood-plates. The cleft through which the plate passes from the red corpuscle can probably close, and the corpuscle still live. Hirschfeld's observations were made on dry preparations controlled by the examination of fresh specimens.

According to Hirschfeld,[38] Engel[39] believes that the blood-plates arise from corpuscles, without the usual biconcavity, and he calls these forms "Blutkügeln." Hirschfeld denies the accuracy of this theory, saying that the red corpuscles from which the plates arise, nearly always have a well marked concavity.

Howell[40] claims there is no connection between the nuclei of red corpuscles and blood-plates, since they do not react the same

toward the triple stain of haematoxylin, eosin and safranin.

Sacerdoti[41] mixed a drop of blood with artificial gastric juice, and observed that the red corpuscles were digested and dissolved in about ten minutes, while the plates and leucocytes at the end of that time, were present in the same number as at the beginning of the experiment. He considers this as proof that the plates are different in structure from the reds.

Hirschfeld[42] opposes the theory that the plates are derived from the nuclear remnants in the red corpuscles, and backs up his objections with the observations made on embryonic blood, on red bone-marrow, and on the blood-forming organs. If the plates came from nuclear remnants, he argues that we should find them in the above situations, but this is not the case. Hirschfeld thinks that the plates which are seen forming inside the red corpuscles, are identical with the "Innenkörper."

The second important theory concerning the origin of the plates, is that they arise from the leucocytes. This view has met with wide acceptance.

Riess,[43] in 1872, advanced the theory, that insufficient nutrition of the blood, caused the destruction of the leucocytes, and thus gave rise to plates. In certain pathological conditions, when the number of leucocytes is increased,[44] he found that there was a corresponding increase in the number of plates. Halla[45] modified the latter assertion of Riess, by showing that while the number of plates and leucocytes respectively, is not necessarily increased or diminished simultaneously, yet under conditions in

which the leucocytes were the most numerous, the plates were also found in greatest abundance, while the red corpuscles were generally decreased in number. Hayem also supports this statement.

Pouchet[46] thinks the plates come from the protoplasm of the leucocytes, since they stain the same. Howlet[47], and Bizzozero[48], working independently, decided that the plates were derived from the protoplasm of leucocytes, because they resemble the processes of the leucocytes, and because, in the dog, they are absent from the spleen, lymph glands, and medulla of the bones.

Hlava[49] supports the theory that the plates are the nuclei of the leucocytes, for the following reasons. First, he has seen white corpuscles filled with plate-like structures. Second, the total number of leucocytes is smaller before coagulation, the multinuclear forms breaking down to form mononuclear forms, and blood-plates. Third, there are more plates in blood examined in salt solution, than in osmic acid, because osmic acid prevents the breaking down of the leucocytes. Fourth, the nuclei of multinuclear leucocytes stain the same, and have the same shape as the plates. Fifth, the fibrin formed from leucocytes has the same microchemical composition as the plates. Sixth, the leucocytes of normal blood break down, thus explaining the presence of plates in circulating blood. Kemp[50], in criticising Hlava's work, brings out the following points: He agrees that the plates are more numerous in salt solution than in osmic acid, but he says this is due, not to the breaking down of the leucocytes, but to the fact that the plates stick to the cover-glass when examined

in salt solution. What Hlava calls plates within the leucocytes, he considers the nuclei of multinuclear leucocytes, because with acetic acid they do not give the same reaction as the plates. He has been unable to confirm the statement that the leucocytes are fewer in number after coagulation, and he has never seen a leucocyte breaking down, although he has seen and figured the formation of plate masses from individual plates, which present an appearance that might easily be mistaken for leucocytes. He shows that plates and leucocytes differ in microchemical reactions, and that although the blood of invertebrates contains no blood-plates, yet multinuclear leucocytes are present.

Prus[67] studied four cases of leukaemia and found in these cases, that there were two million blood-plates, while in normal blood he counted only five hundred thousand. Pizzini[52] studied the blood in croupous pneumonia and the results of his work, as given in his table, show that there is no relation between the number of leucocytes and the number of plates. The plates were increased in several fold, but the leucocyte count varied now in one direction, now in another, probably owing to the fact that the examinations were not made at the same interval after the crisis. Rabl,[53] using Heidenhain's aqueous haematoxylin solution with iron-alum as a mordant and differentiator, finds that the red corpuscles are decolorized sooner than the plates or the leucocytes. We must be cautious about considering this as evidence concerning the different elements of the blood to one another, since, as Rabl himself notes, the individual leucocytes vary considerably in their stain-

ing, and their differentiation is affected by the method of fixation (see page 55). Van Emden[54], opposing this view, claims that we cannot say that the plates necessarily increase in number after the disappearance of a large number of leucocytes, nor can we say that the leucocytes must decrease because plates appear abundantly.

Hirschfeld[55] says that we must admit that rarely, bodies which cannot be distinguished from plates, arise from the leucocytes, although he believes that the greater number of the plates originate from the red corpuscles.

Lilienfeld[56] says that the granular masses formed by the plates, consist of nuclein, while the homogeneous part of the plate is proteid. He is of the opinion that in all probability the blood-plates are derived from the nuclei of leucocytes, and he suggests for them the name. "Nucleinplättchen."

Howell[57], (p. 109-111) begins with the lymphocyte and traces its formation through different forms, to the multinuclear leucocyte, which undergoes disintegration. He is of the opinion that the fragmented nucleus persists for a time as blood-plates, and that the remainder of the cell may go to form the paraglobulin of the blood.

Bremer[58] and Lavdowsky[59] (p. 21) state emphatically that the leucocyte nuclei have nothing to do with the blood-plates.

The third important theory concerning the blood-plates is that they are independent elements. Max Schultze[60] (p. 36), Osler ([61] p.144, [62] p. 530), Bizzozero ([63] p. 17. [64] p. 348). Laker ([65] p. 193), Lavdowsky ([66] p. 64, Halla[67] p. 378),

Schimelbusch (68 p. 100, 69 p. 327), Petrone[70] (p. 934), and Kemp[71] (p. 319), all support this theory.

LÖWITS WORK.

Löwit[72] objected to this claiming that blood-plates were not normally pre-existent in the blood, but that they were a precipitate of globulins from the blood-plasma, or that rarely, they might be derived from the leucocytes. He says that in blood drawn into 20-25% salt-solution,[73] there are no plates, consequently plates do not exist in normal blood, since they would not be dissolved by this fluid. Lowit emphasized especially, that cooling the blood or injury to the blood-vessels would cause this precipitate of globulin (plates). He states that if the mesentery be examined in a bath of warm oil, and the blood-vessels at the same time be subjected to no strain, no plates will be found, whereas, if the temperature falls, or the vessel walls are injured, plates appear.

Woolridge[74] holds a similar view (according to Bizzozero), since he found in the plasma of peptonized blood, a substance precipitated which he calls "A" fibrinogen, and to which he ascribes an important part in coagulation. The microscopic form of this precipitate, he says, is such that it cannot be distinguished from the blood-plates. Bizzozero[75] says of this precipitate that in the same preparation, it is easy to distinguish the granules of "A" fibrinogen from the blood-plates. The latter are pale, granular, and united in masses, with a characteristic reaction toward water, and dilute acetic acid. The former are homogeneous, more highly refractive and are arranged "en chapelet." With dilute acetic acid they become more refractive, while with water they disappear.

PLATES IN CIRCULATING BLOOD

The fact that plates do exist in normal circulating blood has been conclusively shown by Bizzozero[76], Lavdowsky[77], Hlava[78], and Schimmelbusch[79], who have seen them in the uninjured blood-vessels. Schimmelbusch took all the precautions necessary to prevent injury to the mesentery, and to avoid possible changes in the blood due to variations in temperature. The first observation of the plates in the blood vessels had been made some time before this by Osler[80]. He worked on the connective tissue in the back of young rats. Pouchet[81] followed his work with observations on the mesentery of rabbits. Both found blood-plates in the circulation. To make the proof of their existence still more conclusive, Bizzozero[82], Laker[83], and Sacerdotti[84] (p. 43 and 44), found plates in the blood-vessels of the bat's wing, from the very first moment of observation. The plates did not increase in number during the experiment and the wing was held in the natural position for flight so that no lesion could possibly occur, as might have been the case with the mesentery. This work on the bat's wing was done to remove Lowit's objection that exposing the mesentery would cause the leucocytes to break down forming plates. The view of Lowit[85] that plates are precipitated globulins, which at the body temperature are normally in solution in the plasma, has received no additional support.

Lavdowsky[86] (p. 21) says that the blood-plates must be considered as an independent element of the blood. He discusses the possibility of the plates being derived from the nucleoid sub-

stance in the red corpuscle. He says that they differ in structure, and with a mixture of equal parts of iodic-acid and a saturated solution of corrosive sublimate, the nucleoid substance did not appear at all, while the plates showed clearly.

Our own work on the subject of the origin of the blood-plates, may be given as follows.

WLASSOW.

In using Wlassow's[87] paraffine-oil-vaseline mixture we found blood-plates there, although they were not as numerous as when blood is examined in ordinary preserving fluids. The fact that Wlassow says that many of the plates contain haemaglobin when seen in his first solution (see p. 5), and also his statement that the microcytes were not present, makes us believe that he did not distinguish clearly between the microcytes and the plates.

ARNOLD.

Undoubtedly Arnold[88] did see red corpuscles in the process of budding, but we believe that this was due to the 10% K I solution in which the corpuscles undergo an artificial change; because in the first place, if the reds normally undergo such changes we should certainly expect to find them taking place when the blood is observed fresh, or in isotonic solutions; in the second place we should expect to find such forms in all stages of budding, when preparations are fixed in formaldehyde, corrosive sublimate, or osmic acid. There is a difference in osmotic pressure between the K I solution and the blood-plasma sufficient to cause the red corpuscles to become badly crenate. We have never seen red corpuscles giving

rise to buds when observed fresh or in isotonic solutions, and we have never found the different stages of the process in preparations, fixed wet, and not allowed to dry before they are mounted.

Bettmann[89] states that in isotonic and hypisotonic solutions he observed no budding of the red corpuscles, and he was unable to find plates, under these conditions. In hyperisotonic, budding forms were found, as well as a number of blood-plates.

There is some question as to whether Arnold's plate-buds are really blood-plates. Hirschfeld[90] is of the opinion that the appearances seen in Arnold's potassium iodide solution are not all blood-plates. He supports this by showing that part of them dissolve in 1% acetic acid. We agree with Hirschfeld that all the structures described by Arnold as blood-plates, are not true plates. To settle this point we tried the effect of artificial gastric-juice on preparations made according to Arnold's directions. The real plates are digested only slowly, and leave behind them a granular mass, the red corpuscles are digested rapidly leaving no residue, while Arnold's plate-buds, like the erythrocytes, digest at once, and completely. This supports our view that Arnold and his pupils worked on fragments of the red corpuscles. We find further corroboration in the effect of physiological salt solution on the "steckapfel" forms obtained with 10% K I solution. If, after these forms are well developed, we wash out the K I with .75% Na Cl, then we find that the erythrocytes regain their normal size and appearance, without shedding the buds. We believe that this shows conclusively that Arnold's forms are merely the results of an

artificial distortion caused by the difference in osmotic tension. We should also expect to find that these plate-buds were sticky if they were really identical with plates. They are never found sticking to the glass or to the red corpuscles when examined pure in thin layers according to Determann[91]'s sterile tube method. After the buds break loose, forming the alleged plates they are described as floating off free, while the true plates stick to the glass. It is the prevailing view that budding, as well as other distorted forms of the red corpuscles are connected with the dying of the cell, whether this be the result of artificial reagents, or a part of the vital phenomenon.

Many attempts have been made to establish a relationship between the blood-plates and the other elements by their staining reactions. They stain like the red corpuscles with eosin, safranin, and fuchsin, like the nuclei of the leucocytes with the aniline dyes, and methyl-green, while with haematoxylin in weak solutions they appear to stain intermediately between the stroma of the erythrocytes and the nuclei of the leucocytes.

Comparison of the counts given in the tables, shows that there is no fixed relation between the number of leucocytes and the number of plates, or the number of erythrocytes and the number of plates.

In structure the plates are granular bodies containing nuclear matter. The different observers have described the shape of the blood-plates variously, some claiming that they are biconcave, others that they are flat, while still others say that they are biconvex.

The shape depends to a great extent on the fluid in which they are studied. In Bizzozero's fluid, .75% Na Cl tinged with methyl-green or methyl-violet, they are biconcave, while in formaldehyde solutions they are likely to be convex.

The size varies from oval forms, one third the diameter of the red corpuscle, to long sausage-shaped bodies, even larger than the erythrocytes. Indeed, in exceptional cases, large oval or irregular forms have been observed, which approximate the leucocytes both in shape and size. We have never seen haemoglobin in the plates.

We have never been able to trace the stages between the plates and the red corpuscles, although we have worked on the regeneration of blood in young kittens, after severe haemorrhage when such forms would certainly appear in the circulation, if they ever do. We have worked on the bone-marrow before and after haemorrhage, and we have not found plates in the process of formation, although in fresh marrow preparations examined in 2.5% aldehyde, plates were found to be present.

With artificial gastric juice the red corpuscles digest at once, the leucocyte-stroma and the homogeneous part of the plate next, while the leucocyte nuclei and the plate-granules are left undigested.

All of our work, as outlined above, tends toward the conclusion that the plates preexist in the blood as independent elements.

The two most important theories which have been advanced in regard to the function of the blood-plates are: (a) Hayem's haematoblast theory; (b) the theory that they play the principal role

in coagulation.

HAEMATOBLAST THEORY

Hayem[92]'s theory is that the plates pass through various stages culminating in the red blood corpuscles. The gap between the largest plates and the microcytes is filled by forms which he calls, "globules nains" (dwarf globules.) In support of his theory, Hayem shows the many similarities between the corpuscles and the plates. Of still more importance, are the observations which he made on pathological cases. He found in the regeneration after haemorrhage ([93] p. 120), the rise in the number of red corpuscles was preceded by an increase in the number of blood-plates, which increase lasted until about the time that the red corpuscles reached their normal number, after which the plates returned to their average number. He also found that in acute diseases accompanied by an anaemic condition, the blood-plates in many cases, increased in number at about the time when the fever broke. This increase in blood-plates was followed by a rise in the number of erythrocytes. Hayem calls this rise in the number of the red corpuscles, the "haematoblastic crisis." This work was continued by one of Hayem's pupils, Reyne,[94] who analysed the following fifteen cases, and found haematoblastic crises in all of them:

Pneumonia	4 cases.
Erysipelas	1 case.
Scarlet Fever	3 cases.
Measles	1 case.
Gastric Fever. (embarras gastrique febrile)	2 cases.

Typhoid Fever. 1 case.

Metrorrhagia 3 cases.

Riess[95] (-- p. 696) reports on the work of Repac.

Ehrlich[96] (p. 43) disputes Hayem's statement that the microcytes are young forms of the red corpuscle. He considers them as broken down erythrocytes. Obrastzoff[97] (p. 408-410) opposes Hayem's theory on the ground of the difference in the microchemical reactions of the erythrocytes and the plates. He says the latter do not contain haemoglobin.

Afanassiew (p. 250) says that the blood-plates grow, taking on a sheath of protoplasm and acquiring haemoglobin to form nucleated red corpuscles. The nuclei of these corpuscles are extruded and become plates again. This normally takes place in bone-marrow. Drueben[99] (p. 39) says that plates may be haematoblasts but that further researches must be made to settle the point. Leube[100] (p. 653) seems to agree with Hayem's haematoblast theory and Neuman[101] (p. 434) also supports it.

PLATES FORM NUCLEATED REDS.

The theory of the relation of the blood-plates to the coagulation of the blood is still a subject of dispute, although an exceptionally large number of investigators have worked on it. The principal role in coagulation has been ascribed to each one of the three morphological elements of the blood. Hayem[102] (p. 286-287) says that even though blood is examined as quickly as possible after it is drawn, one never sees a white corpuscle breaking down. Kemp[103] agrees

RELATION OF THE PLATES TO COAGULATION.

with both Haye[104] and Bizzozero[63] that conditions and reagents which prevent the breaking down of the plates retard to the same extent the formation of fibrin. During coagulation granular masses are formed. The general view in regard to these masses is that they are formed from plates. This view is held by Schultze[106] (106 p. 36,37), Osler (107 p. 530), Hayem (108 p. 675,704 and 109 p. 215), Leube[110] (p. 654), Bizzozero (111 p. 352 and 112 p. 18), Davidson (113 1028), Laker (114 p. 183), Halle[115] (p. 217), Löwit[116] (p. 297), Schimmelbusch (117 p. 101 and 118 p. 220), and Kemp[119] (p. 321). In blood which has coagulated we observed masses of broken down granular material from which fibrin threads tend to radiate. These masses were formerly attributed by the majority of observers to broken down leucocytes. Hayem[120] first emphasized the fact that they were derived not from leucocytes but from blood-plates. Then followed the work of Bizzozero[121] and a number of others, most of whom traced these granular masses to broken down blood-plates and ascribed to the plates the leading role in coagulation.

Kemp[122] (p. 322) and Fusari[123] (p. 246) notice plates sticking to the edge of leucocytes or red corpuscles, and they call attention to the fact that the fibrin radiates from the plates and not from the leucocytes. This appearance of fibrin threads radiating from the group composed of leucocytes and plates has led many observers erroneously to believe that the leucocyte was concerned in the formation of fibrin.

Bizzozero[124] (p. 301), Laker[125] (p. 25-27), Prus[126] (p. 469), Neuman[127] (p. 439), and Kemp[128] (p. 322) assert that the granular masses

are derived from the plates.

LILIENFELD ON COAGULATION

Lilienfeld[129] is one of the strongest advocates of the theory that leucocytes break down during coagulation. He says (p. 121), "I have repeatedly seen leucocytes breaking down in extra-vascular drops of blood at times when I was not thinking of coagulation, but was simply studying the histological structure of the blood." Lilienfeld admits that the plates break down also. He digested fibrin preparations with artificial gastric juice, and found the nodes of the fibrin net-work to consist of the residue of blood-plates, and the naked nuclei of leucocytes, both of which reacted in the same manner.

Cavazzini[130] (p. 73) made some interesting observations on $K_4Fe(CN)_6$ made up with physiological salt solution. He found that this fluid prevented coagulation, and preserved the blood-plates and leucocytes. By diluting to different degrees he reached a dilution which just failed to prevent coagulation, but at this point the plates broke down although the leucocytes were still well preserved.

Tschistowitsch[131] also studied the question of the breaking down of the leucocytes. He used different substances to mix with the blood, some of which had a globulicidal action on the erythrocytes, and in none of his observations did he see leucocytes breaking down.

Muir[132] (p. 267) says that in the earliest appearance of the fibrin needles, their closest formation is around the altered

blood-plates. Often fibrin filaments can be seen continuous with the spinous processes of the blood-plates, but they never have any relation to the leucocytes. Petrone[135] says that during the first days of poisoning with pyrogallol, the blood clots firmly and rapidly in spite of the fact that the plates are not attacked, but are on the contrary increased. He claims that the fibrin comes from the erythrocytes, which contain fibrinogen. The increase in the number of blood-plates had been noted previous to this by Afanassiew, in 1884.

When Lilienfeld[134] asserted that leucocytes break down, it is probable that he was studying, in fresh preparations, plate masses which have leucocytes caught in them. His method of studying coagulation was to allow the blood to clot between the cover glass and slide. The clot was then washed with water until all the red corpuscles were removed; the preparation was next fixed in Herman's fluid, or in osmic acid, stained and allowed to dry in the air. This method involves two objectionable proceedings. When a blood preparation is washed in water the histological elements are badly distorted. Drying a preparation causes mechanical ruptures as can be shown by comparing preparations which have been dried with those which have not. In addition to these objections great precautions must be taken in separating the cover-glass from the slide when clot has taken place between them, to prevent mechanical violence.

The method of the formation of the clot has already been described by a number of investigators. From our microscopical study

The italics are ours.

of the formation of fibrin we agree with most of the descriptions given. First the plates become granular in appearance, then they collect in masses which are sticky and which closely resemble broken down leucocytes. Fibrin threads are then deposited, a large number of them radiating from these plate masses, although some of them are deposited out in the open field. That these granular masses are not disintegrated leucocytes is shown by the fact that we can watch their formation under the microscope, from individual plates.

DEFIBRINATION EXPERIMENTS.

A method of obtaining reliable information on the relation of the plates to coagulation was suggested by some experiments of Bizzozero[135], in which he drew about one third of the blood from a dog, defibrinated and reinjected it until the blood was deprived of its fibrinogen and refused to clot. He found that after complete defibrination the blood-plates were too few to count. We have called this proceedure <u>fractional defibrination</u>. Bizzozero studied the regeneration of the plates on successive days after the bleeding, but he did not study the effect of each stage in the fractional defibrination. It seemed to us that a determination of the number of blood-plates, red corpuscles and leucocytes together with a record of the specific gravity of the blood would furnish valuable data with regard to coagulation. Therefore a series of experiments was made covering this important part of the field, which has, as far as we know, never been touched on before. A preliminary report on the first few experiments was read by Prof. Kemp before the

American Physiological Association in December, 1900. We have studied three phases of the relation of the plates to the other elements: first, the normal relation; second, their changes during fractional defibrination; third, their regeneration after defibrination.

METHODS OF COUNTING.

The first difficulty met with was the question of obtaining an accurate count of the blood-plates. The most characteristic properties of the blood-plates are, their tendency to become sticky the moment the blood is drawn, and the rapidity with which they break down. Therefore any method which involves the drawing of the blood into a measuring pipette has this objection, that some of the plates are lost by reason of their sticking to the sides of the tube. Many investigators have used this method although they themselves have called attention to this error. The method which we use is a modification of one which was first proposed by Laker[136] and by Kemp[137] independently in 1886. It usually goes by the name of the Laker method. The original method consists in making two counts; the first, for an ordinary count of the red corpuscles, and the second, for a ratio between the blood-plates and the red corpuscles within a given field in the Thoma-Zeiss counting chamber. From this ratio an absolute value for the plates was easily calculated. Van Emden[138] attempted to make a comparison between the Laker method and the pipette method showing that the difference in the counts obtained was within the limit of error, but his results cannot be applied to pipette methods in general, since he worked with a pipette artificially cooled, thus retarding alterations in the blood-plates.

In the accompanying table we have indicated the method used, as well as the fluid employed by the observers who have attempted to make an enumeration of the plates.

OBSERVER	PLATES	PLATES:REDS	FLUID	METHOD	REFERENCE
AFANASSIEW	180000 TO 300000		.6% NaCl. .6% Dried Peptone in H_2O Tinged with Methyl-Violet. A little Carbolic Acid	Pipette Method	Arch. f. klin. Med. 1884 Vol. 35 Pg 217
BIZZOZERO	200'000	1:20.	1% Osmic Acid ... 1 pt. .7% NaCl: ..... 3 pts Methyl. Violet ... 1% Mg. SO_4 Methyl Violet	LAKER Method	Zeit. f wiss. Mik. 1892 Vol. 9 Pg 231
BRODIE AND RUSSELL	635'300	1:8.5	Na Cl. 2% .... 1 pt. Glycerine Sat with Dahlia .... 1 pt	LAKER Method	Jour. of Physiol. 1897 Vol 21 Pg. 390-395
DETERMANN		1:18.0 TO 1:30.0 AV. 1:22.0	1% NaCl. with 5% $K_2Cr_2O_7$* ?	LAKER Method	Deutsch. f. klin Med. 1898 Vol. 61 Pg 365
FUSARI	180'000 TO 250'000		1% Osmic Acid ... 1 pt. .75% NaCl. .... 1 pt. Methyl Violet ..... 1% Osmic Acid ... 1 pt. Na_2SO_4 (Sp. Gr. 1025) 1 pt. Methyl. Violet	LAKER Method	Arch. per le Scienze Med. 1886 Vol. 10 Pg. 235
HAYEM	255'000 Varies with the time of day		NaCl 1 gram; Na_2SO_4 5 grams; $HgCl_2$ .5 grams; Dist. Water 200 c.c.	Pipette Method	Du Sang. Paris 1889
KEMP AND CALHOUN	862'000 TO 833'000	1:5.6	1% Na Cl 15 pts; Formol 1 pt; Methyl Green	LAKER. Method	Am Jour. of Physiol. 1901 Vol 5 Pg IV.

* determinations of value made of this period but prepared [illegible]

OBSERVER	PLATES	PLATES:REDS	FLUID	METHOD	REFERENCE
LAKER	Counted Blood of Guinea Pigs			LAKER Method	Sitz.B. d. k.k. Akad Wien Vol. 93, 1886 Pg 32
MUIR	200'000 To 250'000		Na_2SO_4 (Sp.Gr. 1022) tinged with Methyl-Violet	Pipette Method	Jour of Anat. & Physiol. 1890 Vol. 25 Pg. 269
PETRONE		Found Ratio of Leucocytes To Plates	See Prus	Pipette Method	Cent. f. innere Med. 1895 Pg 638
PIZZINI	300'000 To 350'000	1:15.5	1% Osmic Acid with Methyl-Violet	LAKER Method	Riforma Medica 1894 Vol 2 Pg 374-379, 386-391
PRUS	500'000	See Petrone	Osmic Acid 1% ---- 10 c.c Chromic Acid 1% --- 10 c.c. Glacial Acetic --- 1 c.c	Pipette Method	Medycyna 1886 No 39, 40.
RABL				Ratio in Dry Preparations	Wien klin. Wochen. schr. 1896 Vol 9 Pg 1060-1062
SACERDOTTI					Arch. per le Scienze Med 1893 Vol 17
SALVIOLI	Rabbits 175'000 Guinea Pigs 213'000 Dogs 207'000		1% Osmic Acid 1 pt. .7 Na. Cl. 2 pts. Methyl-Violet	LAKER Method	Virch. Arch 1891 Vol 125 Pg 378
VANEMDEN	245'000	See Petrone	See Prus	LAKER Method Cold Pipette Method	Fortschr. der Med. 1898 Vol 16 Pg 241-251

COUNTING FLUIDS.

The question of a perfect preserving fluid is one of utmost importance in obtaining an accurate count of the blood-plates. The fluid must be one that will absolutely prevent important changes occurring in the plates, and yet it must be a fluid that will keep them from sticking to the cover-glass and which will allow them to move freely in the field; at the same time the red corpuscles must be preserved in their normal condition. We have tested the action of the following, counting fluids which have been recommended by previous authors.

Bizzozero[139]'s original fluid:-

.75% Na Cl tinged with methyl-violet.

We have found that although this preserves the plates for a time, after thirty minutes the accuracy of the ratio cannot be depended upon.

14% $Na_2 SO_4$

This was also suggested by Bizzozero and although it preserves the plates, buds are shed from the reds.

Hayem[140]'s fluid:----

$Hg Cl_2$	.5 grams
Na Cl	1. gram
$Na_2 SO_4$	5. grams
Distilled $H_2 O$	200 grams

The chief objection to this solution is that it precipitates the albumin and some of this precipitate might be confused with the plates.

Brodie and Russell[141]'s solution:---

Na Cl 2%	1 part
Glycerine saturated with dahlia	1 part

As Brodie and Russell claim, this solution preserves the plates and allows them to move freely in the field although it has the disadvantage of decolorizing the red corpuscles in a short time. We have found that the color is much too dense to work with conveniently, and the decolorization brings in a possible error which may be avoided by the use of formaldehyde solutions.

With Müller's fluid and potassium bichromate solutions (6%, 4%, and 1%) the red corpuscles are well preserved, but after a short time granules formed which may easily be confused with the plates.

Prus[142]'s fluid:----

Osmic acid 1%	10 c.c.
Chromic acid 1%	10 c.c.
Glacial acetic acid	1 c.c.

This is a modified Flemming's solution which has been used by Van Emden and by Petrone[145] as well as by Prus. It cannot be used to obtain the ratio of the plates to the red corpuscles since all the reds are decolorized by it.

We have tried various percents of osmic acid with salt solution and although it is a perfect preservative it is extremely difficult to regulate the proportion of fluid and blood so that the red corpuscles are not clumped making it impossible to determine the ratio.

OUR COUNTING FLUID. We found a solution of our own which has the following formula to be very satisfactory.

Formaline	10 c.c.
Distilled Water	150 c.c.
Sodium Chloride	1.2 grams

This gives a 2.5% solution of formaldehyde. When the aldehyde solution was used without the addition of salt, we found that the red corpuscles were completely decolorized in a short time. The use of salt tends to offset this decolorizing effect of the aldehyde. About equally good results are obtained when the Na Cl is present in from .75-3.%; above 3% the reds become crenated. In the course of our work we found one specimen of this solution which decolorized the red corpuscles. We suspected this decolorization to be due to acid formed by the oxidation of the aldehyde. On testing with litmus it was found to be slightly acid. This led us to make some experiments on solutions containing varying amounts of formic acid. If to the 2.5% formaldehyde containing salt, we add enough acid to make a 1% solution, the red corpuscles are clumped and decolorized. The action on the leucocytes and plates was similar to that of 1% acetic acid. The same formaldehyde mixture with enough acid to make a .1% solution decolorized the red corpuscles almost immediately. With a .01% acid solution the reds were not clumped, although they were slightly swollen and no longer biconcave. They retained their color six hours. We found that salt was always necessary to preserve the haemoglobin in the red corpuscles. Our results were as follows:--

Aldehyde	Acid	Salt	time of decolorization.
2 1/2%	.1%	none	immediately
2 1/2%	.075%	none	immediately
2 1/2%	.05%	none	immediately
2 1/2%	.025%	none	immediately
2 1/2%	.001%	none	immediately
2 1/2%	.1%	.75%	immediately
2 1/2%	.075%	.75%	immediately
2 1/2%	.075%	1.50%	15 minutes
2 1/2%	.05%	.75%	2 minutes
2 1/2%	.025%	.75%	no change in 1 hr.
2 1/2%	.001%	.75%	not decolorized

This shows conclusively that small amounts of formic acid (.025%) have no effect on the efficiency of the counting fluid. The figures given for our solutions refer to absolute percentages of formic aldehyde.

The proportion of blood to the fluid does not have any effect within wide limits; if the amount of blood be large and the amount of aldehyde quite small, precipitates, probably of albumin, are formed. The dilution is so regulated that the field is not enough crowded with red corpuscles to mask any of the plates. The aldehyde solution will preserve the blood-plates indefinitely. After our work was well under way we found a reference to the paper of Marcano [504] in which he recommends this solution:---

Na_2SO_4 (sp.gr. 1020)	100 c.c.
Formol	1 c.c.

We tried this fluid and found it most excellent for counting plates,

but it does not give better results than the solution of 2 1/2% formaldehyde made up with salt.

OUR METHOD

We employed a modification of the Laker method for determining the number of plates. The red corpuscles were counted by means of the haematocrit, following Daland's method of diluting the blood with an equal volume of 2.5% $K_2Cr_2O_7$ to prevent its clotting. The $K_2Cr_2O_7$ was first measured in a capillary pipette and blown out into a small watch glass; the same amount of blood was then drawn up into the pipette and blown out into the watch glass containing the bichromate solution and mixed. This mixture was sucked up into the haematocrit tubes and centrifugalized for five minutes in a Bausch and Lomb haematocrit driven by a small electric motor at the rate of about 2000 revolutions per minute. We found this method to be thoroughly reliable as compared with the Thoma-Zeiss counting apparatus.

The ratio between the erythrocytes and the blood-plates was determined by means of the Thoma-Zeiss counting chamber.

The leucocyte count was made in the regular way with the Thoma-Zeiss counting apparatus, using 1% acetic acid to decolorize the reds. No attempt was made to distinguish the different kinds of leucocytes.

The specific gravity was taken according to Hammerschlag's benzol-chloroform method, and a Mohr-Westphal balance was used to determine the specific gravity.

All these observations were made for three days before the blood was defibrinated, the blood being taken from a stick in the dog's ear which was shaved before each observation. The count of

the reds and the leucocytes, the specific gravity and the ratio of the plates to the reds, were all made from a specimen of blood flowing freely from the same wound. For the defibrination experiment the dog was first put under chloroform and the anaesthesia was then continued with ether. The blood was drawn from the femoral artery, one third of the total blood being drawn each time. It was whipped with a bunch of wires until all the fibrin was formed, it was then filtered through two folds of cheese-cloth. The temperature was maintained at about 37° C by keeping the blood in an evaporating dish which was surrounded by warm water. This defibrinated blood was then injected into the femoral vein, allowed to circulate for a varying time (average about 4 minutes) after which a count was made. This process was continued until the blood refused to clot. Usually eight defibrinations sufficed to rid the blood of its fibrin. The blood-vessels were then ligated. In every case the number of plates was greatly diminished during the defibrination. The leucocytes were also decreased during some of the operations but a count taken from the defibrinated blood before and after it was filtered through the cloth, showed there was some loss during filtration. A count made before filtering showed few or no plates thus proving that their decrease could not be due to their being caught in the filter. The arterial canula was always cleaned out and thoroughly dried with filter-paper each time before the blood was drawn for the count.

CONCLUSIONS DRAWN FROM DEFIBRINATION EXPERIMENTS.

Our results are shown in the accompanying tables. In all cases except dogs No.13, 15 and 16, there was a progressive decrease in the number of plates with each defibrination.

This decrease extended through the first six defibrinations as may be seen by examination of the composite curve.

This part of the curve is made from the data obtained from fourteen different experiments. This seems to show conclusively that the plates are directly concerned with coagulation.

The leucocytes vary so greatly under normal conditions that it is more difficult to judge from numeration experiments on them as to what part they play. It may be said, however, that there is no considerable progressive loss, such as is noted for the plates. In some cases (see dogs number 3, 5, and 14) the leucocytes seem to show a decrease in number; in most cases there is a decrease early in the operation, followed by an increase which may be great enough to make the number higher at the end than at the beginning. We have counted the colorless corpuscles in order to determine whether there was a distinct and regular variation in them during defibrination. There was none.

The red corpuscles were not as a rule materially decreased in number during the operation but on the following day there was a diminution in the number of reds, which diminution increased progressively for several days, reaching its maximum usually about the third or fourth day; after which the number of reds rose irregularly, in some cases not reaching the normal in fifteen days.

The specific gravity varied within narrow limits during defibrination. There was a slight tendency to decrease. On the following days, however, the decrease was striking. It may be said

that this was coincident with the fall in the number of the red corpuscles. The strict relation between the reds and the specific gravity which has been claimed by other authors has not been found to hold good throughout our experiments; the number of exceptions being so numerous and so marked as to lead us to doubt the advisability of estimating the number of reds from the specific gravity, as a general rule.

WORK OF ARTHUS

In addition to the fractional defibrination experiments we performed two others along a line suggested by the work of Maurice Arthus.[195] He drew considerable quantities of blood at intervals of several minutes obtaining the blood each time from a different artery. He noted the time of clotting for each sample and found that the blood coagulated more quickly in the last samples than in the first. For determining the time of clot, the blood (about 10 c.c.) was drawn into a test-tube and as soon as the blood was firm enough to allow the tube to be inverted, the time was taken. The test-tubes used for this determination were all of the same diameter. In our experiments we have counted the number of the red corpuscles, of the plates, and of the leucocytes, in addition to taking the clot time and the specific gravity. We thought perhaps there would be a variation in the number of plates which would explain the difference in the time of clot. Our experiments are too few in number to draw conclusions. The data is as follows:-

Time	Source	Am't Drawn	Reds	Whites	Sp.Grav.	Plates	Clot Time
3:47	L.FEM.	10 c.c.	4,100,000	11600	1052.	313,000	6 min.
----	L.FEM.	60 c.c.	----------	-----	----	-------	------
4:12	L.CAR.	10 c.c.	4,000,000	10600	1048.5	265,000	6 min.
----	L.CAR.	60 c.c.	----------	-----	------	-------	-----
4:25	R.CAR.	10 c.c.	2,800,000	11600	1044.4	243,000	1m.40s.
5:00	R.FEM.	10 c.c.	3,000,000	-----	1042.6	285,000	2 min.
4:32	R.FEM.	10 c.c.	5,200,000	13500	1056.2	619,000	4 min.
4:35	R.FEM.	140 c.c.	----------	-----	------	-------	-----
4:59	L.FEM.	10 c.c.	5,200,000	9600	1054.2	684,000	8 min.
5:26	R.CAR.	10 c.c.	4,000,000	13900	1052.0	384,000	7m.30s.
5:33	R.CAR.	140 c.c.	----------	-----	------	-------	2 min.
5:49	L.CAR.	10 c.c.	4,800,000	12300	1048.	659,000	3 min.

After the dog was anaesthetized the two femoral and the two carotid artieries were laid bare, but the canulae were inserted only just before the blood was to be drawn. This was done because Arthus says that if the blood stops circulating and is dammed up at the end of the artery, the clotting time for this blood is affected. It was drawn from a different artery each time that the count was made in order to obtain blood which had just been in circulation and to avoid the influence of parts of clot left in the canula from the previous specimen. Our results are too few to be of value as they stand, but we offer these experiments with the hope that sometime they may be extended and improved in such a way as to give valuable data on the subject of coagulation.

METHODS OF FIXING BLOOD PREPARATIONS

Various attempts have been made to determine the composition of the blood-plates and their relation to the other elements by means of their staining reactions. Most of the stains are mechanical, that is to say, they do not enter into a chemical combination with the protoplasm of the cell. Fischer[46] states that methyl-green used in acid solution is the only true chemical stain. The staining reactions are materially affected by the method of fixation. In our work we have followed Fischer's outline of fixatives in making preparations for staining. The ordinary fixing reagents for blood are solutions of corrosive sublimate, osmic acid, potassium bichromate, alcohol, and formaldehyde.

Corrosive sublimate used in saturated solution preserves the elements of the blood very well, but it has this great objection: All of the proteids in the blood are precipitated by it, and this precipitate is insoluble in water and in alcohol. Our method of using $HgCl_2$ was as follows: A small drop of blood fresh from the wound was smeared between two clean cover-glasses which were then dropped, preparation side down, into the fixing reagent without allowing them to dry. The manipulation must be as rapid as possible in order to prevent the blood-plates from undergoing change. The corrosive sublimate solution fixed the corpuscles firmly to the cover-glass, but the film of precipitated proteid could still be distinguished in the field, and it interfered with finer observations on such delicate histological structures as the blood-plates. Platinum chloride has the same effect as corrosive sub-

linate, but it may be used in very dilute solutions. The cost of this fluid is against its extensive use.

Osmic acid causes no permanent precipitate when used on blood preparations. It causes the elements to adhere firmly to the cover-glass and gives very clear fields. When we desired a preparation which contained but a few red corpuscles we touched the cover-glass to the drop of blood moving it quickly around until a thin film of blood was spread over the surface. It was then quickly passed through .75% Na Cl before throwing it into a watch-glass of 1% osmic acid to fix it. This washing in salt solution removed most of the reds while the plates were left sticking to the cover-slip where they were fixed by the osmic acid. Osmic acid vapor has the same fixing power as the solution but it is unavailable for our purposes, since the preparation dries. Solutions of equal parts of osmic acid 1% and $K_2Cr_2O_7$ 5 % gave a precipitate over the field. The influence of fixation in osmic acid on the staining reactions will be taken up in detail later.

The granules precipitated from the blood by weak solutions of $K_2Cr_2O_7$ have already been mentioned. This precipitate is completely insoluble in water. Bichromate solutions are seldom used pure.

The chief objection to the use of alcohol as a fixing and hardening reagent for blood, is that the preparations must be dried if we are to obtain the blood elements undistorted. We have used 95% and absolute alcohol on wet preparations and in every case in addition to the distortion we found that the field was covered with a precipitate. The same objection applies to

the use of alcohol and ether. For dry smears we have found that alcohol and ether, and absolute alcohol give excellent results. We have preserved our specimens fixed by other methods by running them up the grades of alcohol to 95%.

Formaldehyde is one of the most generally useful fixing and hardening reagents for blood. A thick smear may be dropped directly into the mixture and although it preserves the red corpuscles perfectly, yet they are not fastened firmly to the cover-slip. This proves very advantageous for obtaining specimens showing a large number of plates. A few red corpuscles stick to the cover-glass, making a comparison possible. The blood film may be fastened to the cover-glass more firmly by passing the preparation up through the grades of alcohol. The formaldehyde solution used was either 2.5% or 5.% and it always contained .75% Na Cl. The length of time the specimen was left in the aldehyde solution varied from 30 minutes to 24 hours without causing any change in the preparation. Under ordinary circumstances there is no precipitate formed with solutions of 10% and under, but with 40% aldehyde some of the proteids are precipitated in insoluble form.

Combinations of formaldehyde with alcohol have been recommended. Marcano[147] used 5% formol in alcohol. When used on wet preparations, the corpuscles are badly distorted, and there is a precipitate formed. When used on dry smears, as Marcano used it, beautiful results are obtained. Japha[148] uses a 2 1/2% solution of formol in alcohol for fixing dried preparations. These solutions have the advantage of fixing rapidly, a minute is sufficient.

Like osmic acid vapor, the vapor of formaldehyde may be used to fix blood preparations. Marcano[149] says (p. 764) that in 15 minutes the red corpuscles are fixed so that they will withstand 20 minutes washing, but the blood serum is precipitated and withstands 15 minutes washing. He says that strong formol fixes without this objection but the red corpuscles will no longer take a stain. Benario[150] recommends the following:--

Eosin (concentrated H_2O Sol.)	90 c.c.
Formol	10 c.c.

We have never succeeded in getting an eosin stain when used in combination with formaldehyde.

Marcano[151] has also recommended a formol-eosin solution using 1% eosin in 4% formol. There is not enough formol in this mixture to fasten the blood elements firmly to the cover-glass when used on fresh smears. When a drop of blood is drawn into a small amount of this mixture, all of the blood elements are beautifully preserved, but the red corpuscles, the plates and the leucocytes would not take the stain even when enough eosin was added to make a 5% eosin solution.

We have mentioned the fact that dried preparations are not suitable for our work. Under dried preparations, we class all those blood films which have been allowed to dry anywhere in the process, before they are mounted. It is possible to make dry blood smears so rapidly that the blood-plates may be seen in a fairly good state of preservation. The manipulation and the drying must be very rapid to obtain plate forms at all. The rapid dry-

ing is almost certain to cause mechanical rupture in the blood elements and we believe that many of the appearances described by various observers, notably Lilienfeld[152] who worked on dry preparations to be due to this mechanical rupture. Where dry blood films contain plates it is possible to study their staining reactions as compared with those of the other elements, although in every case we would recommend wet preparations for histological study.

There are but four stains which it is claimed will give any clue to the composition of the blood-plates, the eosin stain for haemoglobin; the Shakespeare-Norris[153] stain for haemoglobin, Macallum's[154] test for phosphorus (nucleoproteid) with nitro-ammonium molybdate, and the acid methyl-green test for nuclear matter. Other stains have also been recommended for the purpose of determining the relation between the plates and the other elements of the blood. We report our results with a number of such stains, though we do not feel assured that they show more than accidental resemblances or differences.

METHYLENE BLUE AND EOSIN.

The most generally recommended and highly praised stain for blood preparations are combinations of methylene-blue and eosin used either as separate stains or in combination.

The following observers have worked with methylene-blue and eosin. Hirschfeld[155], Michaelis and Wolff[156], Japha[157], Willebrand[158], Becker[159], Engel[160], Bremer[161], Jenner[162] and Maximow[163]. Dominici[164] used this combination with the addition of Orange G.

Hirschfeld[165] fixes his preparations with dry heat at

110° C for from 5 to 30 minutes; and then stains them with a 1/2% solution of eosin in 60% alcohol for 1 minute. He follows this by a 1:150 solution of methylene-blue "B Pat." for 15 minutes, then washes, dries and mounts. We found that it was difficult to get an eosin stain and the red corpuscles had a curious spotted appearance. We were unable to find any reference to Methylene-Blue "B Pat." and we used medicinally pure methylene-blue (Grübler). This drove out the eosin and stained the whole field diffusely.

Michaelis and Wolff[166] used preparations fixed by dry heat at from 105°-110° C for 1 hour, then stained in Romanowski's[167] methylene-blue-eosin. They speak of this stain as standing alone in the clearness with which it distinguishes the different kinds of granules in leucocytes and in blood-plates. The action depends to a great extent upon the age, the kind, and quality of the methylene-blue used. The staining power depends on a decomposition product of methylene-blue, which is the essential factor in Unna's polychrome methylene-blue stain. Michaelis identified this decomposition product with Burnthsen's methylene azure. It is always found when methylene-blue in solution is kept for some time, with or without alkali. We are interested in this stain only as it affects the blood plates. They claim the plates are always stained red and show a finely granular structure, "wie mit keiner andere Methode."

One disadvantage of the method is that precipitates of the stain cannot always be avoided, but since these are amorphous, diffuse, and scattered over whole areas of the preparation, they

may be easily distinguished from the blood elements. The stain consists of

2 c.c. azure-blau solution

10 c.c. H_2O solution of Eosin 1:1000

mixed just before using. The precipitate is disregarded and the preparations are stained for 15 minutes, washed vigorously with a stream of water and dried. We have found that the plates stain very faintly if at all with this mixture. The action is not uniform and the precipitate is a very serious objection. It will work for smears fixed, by heat, and by alcohol and ether.

Japha[168] stains dry preparations fixed with formol-alcohol, first with a strong aqueous solution of eosin, and then with a weak solution of methylene-blue. We have not tried this solution.

Willebrand[169] discussed the effect of the reaction on the staining of the blue and the eosin, in the methylene-blue-eosin. In alkaline solutions the blue stain works best; with acid reactions the eosin takes hold. He recommends mixing equal parts of a 1/2 % solution of eosin in 70% alcohol, and a concentrated aqueous solution of methylene-blue. Enough acetic acid is added to make both stains effective (10 to 15 drops per 50 c.c.). The stain is filtered before using. The preparation to be stained may be fixed with absolute alcohol, dry heat or 1% alcoholic solution of formol.

Becker[170] uses the precipitate from Willebrand's stain dissolved in acetic acid.

Engel[171] uses the following eosin solution:--

Eosin	1 gram
Distilled Water	90 grams
Absolute Alcohol	10 grams

The preparation is stained in this for five minutes, then washed well and stained in either a concentrated aqueous solution of methylene-blue or in Lőeffler's methylene-blue. It is important not to overstain with the blue. The blood was fixed by passing the preparations three times through the flame of a Bunsen burner, or by treating with absolute alcohol.

Bremer[172] uses dry preparations heated to 120° C and stained with an aqueous solution of methylene-blue-eosin. He found blue-stained plates being extruded from the red corpuscles.

Jenner[173] uses a blood fixative and stain that consists of equal parts of a 1.2-1.25% solution of Grübler's water-soluble eosin (yellow shade) and a 1% solution of Grübler's medicinally pure methylene-blue. These are mixed in an open dish, stirred with a glass rod, and filtered at the end of twenty-four hours. The residue is dried at 55° C. This is then washed with distilled water and again dried. For use, 5 grams are shaken up with 100 c.c. pure methyl alcohol. It is used as a fixative stain and keeps remarkably well. We have not tried this method.

Maximow[174] studied dried preparations heated to 120° C for two hours. The temperature was allowed to rise and to fall slowly so as to prevent cracking. He says that drying causes the least chemical change. He stains for from 15-30 minutes in a 2-3% aqueous solution of eosin; then washes rapidly in distilled water and

follows this with Loeffler's methylene-blue for from 5-10 seconds; then washes rapidly and dries the preparation with filter-paper before mounting. The Loeffler's solution must be from 5-30 days old. The peripheral zone of the red corpuscles is always lightly colored. We found that with frog's blood this stain gives excellent definition to the nuclear matter, but we also found that in spite of all the precautions taken, the corpuscles were often cracked by the dry heat.

Methylene-blue-eosin combinations may be very useful in distinguishing the different granules in the leucocytes, but for blood-plates they have no value since the plates stain with one combination, red, with another blue, and with a third violet. In other words, with one stain they resemble the red corpuscles, and with another the nuclei. The stain appears to be mechanical rather than a genuine microchemical reaction. Methylene-blue and eosin are not a success when used on wet preparations, since one stain discharges the other.

Dominici[175] gives the following solution:--

Orange G (Grübler)	1 gram
Eosin W. (Grübler)	1 gram
Distilled water	200 grams

He stains first, with this mixture, then washes in 60% alcohol until the excess of the acid stain ceases to dissolve. The counter stain is:--

Toluidin Blue (Grübler)	1 gram
Distilled water	200 c.c.

After the counter stain the preparations are decolorized in 60% alcohol

until the color changes from blue to violet. He completes the decolorization with absolute alcohol and mounts in xylol balsam. We have not tried this method owing to the complicated method of fixation with iodine vapor.

We have found a new mixture of eosin and methyl green with which we have some results that are as good as those claimed for the combination of methylene-blue and eosin. We prepared the stain in two solutions used as follows:--

Solution A.

Methyl green (Grübler)	1 gram
Na Cl	.75 gram
Distilled H_2O	100 c.c.

Solution B.

Eosin (wasserloslich)	1 gram
Water, Distilled	100 c.c.

Take three parts of solution A and two parts of solution B and mix. After mixing allow the solution to stand for at least six hours. Filter and use the filtrate for staining. In dried preparations of frog's blood the nuclear matter was well differentiated, the nucleus being decided a decided green, while the stroma was rose color. We expected to be able to use this stain somewhat extensively, but finding the color was extracted by passing through the graded alcohols, and that therefore the preparation had to be dried before mounting, we gave it up.

MUIR'S METHYL BLUE AND FUCHSIN.

Muir[176] uses a variety of stains on blood, the most highly recommended of which is a "methyl" blue and fuchsin stain. He uses for this, dried film preparations which

are stained for thirty seconds in a saturated absolute alcohol solution of "methyl" blue, washed and dried, then stained in a 5% absolute alcohol solution of fuchsin for the same length of time. He says that the methyl blue stains the nuclei a brilliant hue and the fuchsin stains the reds crimson, while the plates are intermediate. He claims that a weak alcoholic solution of eosin may also be used as a contract stain. Muir must have meant a saturated absolute alcohol solution of methylene-blue, since ordinary methyl blue is insoluble in absolute alcohol. We therefore used methylene-blue.

Our results with the fuchsin and methylene-blue were as follows:--

When the preparations were not dried between the stains, one stain discharged the other making it impossible to get a contrast. Preparations fixed with dry heat, or alcohol and ether, stain well; in frog's blood the nuclei of the reds are blue, the stromata crimson, and the leucocyte nuclei pink; and in human blood the red corpuscles are yellowish red, the leucocyte nuclei purple, and exceptionally distinct, and the plates crimson. With preparations fixed in $HgCl_2$ that have been run up through the grades of alcohol and dried, then stained according to Muir's directions, we find that the stain is too diffuse and that the fuchsin does not take readily. When the preparations are fixed in aldehyde, hardened in the graded alcohols and stained by this method, the stain is very uneven; with frog's blood the stromata are unstained while in the same preparation, the nuclei of the reds stain sometimes with

the fuchsin and sometimes with the methylene-blue. With preparations hardened in osmic acid and dried, then stained in methylene-blue and fuchsin, the staining was not regular throughout. Great parts of the field showed a sharp distinct contrast in the staining of the elements, while in other parts the stain did not act at all. We are at a loss to explain this since all parts of the field were subjected to the same process. With osmic vapor, the fuchsin did not take at all, and the whole corpuscle was stained a diffuse blue. With preparations hardened in osmic acid and potassium bichromate, we have smudgy films with extremely irregular staining.

Muir recommends safranin in alcoholic solution for films fixed wet for half an hour in Hayem's or Flemming's fluid. We found that the stain was pale and diffuse and a counter stain was needed. He also recommends staining films fixed in Hayem's fluid with gentian violet (1:800) for twenty minutes. This shows the plates distinctly.

Muir also worked on methylene-blue and eosin using a saturated absolute alcohol solution of methylene-blue, on blood films fixed in Hayem's or Flemming's fluid then counter staining with an alcoholic solution of eosin (1:1000). He states that it is hard to retain the blue stain with eosin. In aqueous solutions it is impossible to get counterstains with eosin if the methylene-blue be used first. In alcoholic solutions even though they be dried before staining, the eosin tends to decolorize the blue completely and the preparations are very irregularly stained. For further discussion of methylene-blue see page 50 .

Muir says that a saturated aqueous solution of methyl green is a beautiful nuclear stain when used for about one hour on films fixed in Heyen's or Flemming's solution. For our discussion of methyl green see page 58. He seems to think that the methyl blue solution is far more generally useful as a nuclear stain than methyl green.

RABL'S WORK ON BLOOD-PLATES

Rabl[177] uses the stain employed by Heidenhain to demonstrate the centrosomes. He gives his results as follows: In dried preparations of blood the centrosomes of leucocytes do not appear because the latter contract into their spherical form and the centrosomes cannot be seen in the mass. On the contrary the plates stain intensely. The red corpuscles can be completely decolorized, appearing grayish yellow and the plates show a dark blue stain. His method is to make dry smears and fix them with .75% salt solution saturated with $HgCl_2$, for from 15 to 30 minutes. The preparations are then washed well and put in the following iron solution:--

1/2% solution of iron alum

or, Liquor ferri-sulf-oxydate diluted with an equal volume of water. Rabl evidently prefers the latter solution. The film is left in this fluid for one hour and is then put into a .5% aqueous solution of haematoxylin for one half to one hour. All the blood elements should now be stained blue black. The preparations are differentiated in a very dilute solution of the iron salt. At the end of one fourth to one minute the preparation has the same grayish yellow color that it had before treating with the haematoxylin. It is now ready to be washed and mounted. If a contrast stain for

the red corpuscles is desired, any acid aniline dye may be used. Rabl suggests picric acid or aurantia. We found that with wet preparations fixed in $HgCl_2$ for 30 minutes, Rabl's stain worked as well as it did on dry preparations made according to his directions. Wet preparations fixed in aldehyde and passed through the graded alcohol show the nuclei more deeply stained, but the stromata would not decolorize completely. When wet preparations fixed in mercuric chloride were passed through the graded alcohols the nuclei and plates decolorized first, leaving the stromata brown. The same is true of preparations fixed in osmic acid, and in osmic acid and potassium bichromate. With Rabl's stain we found that the decolorization was very irregular and hard to control; that it did not work for preparations that had been in alcohol or alcohol and ether, even though they had been previously fixed in osmic acid, formaldehyde or sublimate. Rabl's stain is of no value to determine the chemical composition of the plates since the staining depends entirely on the length of time the preparation is left in the decolorizing fluid, and on the preserving fluid used.

We will now take up a consideration of the stains which have been given as specific microchemical tests for constituents possibly existing in the blood-plates.

TESTS FOR HAEMOGLOBIN

The stains which have been recommended as tests for haemoglobin are (a) eosin, and (b) the Shakespeare-Norris stain. For a discussion of mixed stains containing eosin, see pg. 49. Eosin is the stain most frequently used in histological work for the detection of haemoglobin. Under favorable circumstances it gives a characteristic

rosy-red color to structures in which haemoglobin is present. We have found that this eosin-reaction depends to a great extent on the method of fixation. With certain fixatives it gives good results and after others it will not work at all. The best results are obtained after fixation with dry heat, alcohol and ether, corrosive sublimate, bichromate of potash (Müller's fluid) and osmic acid. After formaldehyde we have not been able to get satisfactory stains, although we tried the aqueous and alcoholic eosins and the French eosin (all made by Grübler). In most of our preparations the blood-plates when stained with eosin resemble the protoplasm of the leucocytes rather than the red blood corpuscles. This is against the supposition that the blood-plates contain haemoglobin, as claimed by Hayem.

We have also tried the stain recommended by Shakespeare and Norris which is a modification of an earlier method.

MACALLUM'S TESTS FOR HAEMOGLOBIN.

We employed this stain as recommended by Macallum[178] in his researches on the origin of haemoglobin. In this method two solution are used;

Sol. A.	Carmine	2 grams
	Borax	8 grams
	Dist. H_2O	100 c.c.
Sol. B.	Sulphindigotate of soda	8 grams
	Borax	2 grams
	Dist. H_2O	100 c.c.

The borax is ground up in a mortar with the dye, then the water is added and the mixture is allowed to stand for 5-7 hours

before filtering. Solution B. does not keep well, and so it should only be made up in small amounts. Equal parts of solutions A. and B. are mixed just before using and a preparation is allowed to remain in this mixture 15 minutes. It is then transferred to a saturated solution of oxalic acid, after which it is washed, dehydrated with alcohol, cleared in xylol and mounted in balsam. Macallum says that when Erlicki's[179] fluid is combined with indigo-carmine it gives a sure means of determining the presence of the pigment, and that often a grass green net work shows in the carmine red nucleus of a frog's red corpuscle. This grass green color is the test for haemoglobin. He recommended osmic vapor as a fixing reagent because the grass green stroma of the erythrocytes is so distinct after it. With Hg Cl_2 preparations this stain gives, according to Macallum, a deep blue green when haemoglobin is present, while the nuclear matter is red.

We tested this method on the corpuscles of frog's blood in order to obtain a convenient contrast between the stroma of the reds with its haemoglobin content, and the nucleus. We found that after fixation with corrosive sublimate there was a distinct blue color in the stroma of the red corpuscles and the shade of blue depended on the length of time the preparation was left in the oxalic solution. With osmic acid we obtained the green color although we were not able to obtain as distinct a stain as Macallum claims. With Flemming's fluid, osmic and bichromate, and formaldehyde fixation there was no stain. The variability in the action of this indigo-carmine stain after different fixatives as well as with the

oxalic acid solution shows that it cannot be regarded as a strict microchemical test for haemoglobin. We never obtained a satisfactory stain of the plates with it.

MACALLUM'S TEST FOR PHOSPHORUS

Macallum's[180] test for phosphorus consists of staining preparations, in a solution of nitro-ammonium molybdate, for from 18 to 36 hours, and then washing them with distilled water before putting them in a 1% to 4% solution of phenyl hydrazine for from 1 minute to 2 hours. The molybdate solution was made by dissolving one part of pure molybdic acid (MoO_4) in four parts of strong ammonia, then adding slowly fifteen parts of nitric acid (specific gravity 1.2). The proportions indicate weights. Since phosphorus is present throughout the cells, in the form of lecithin, we extracted the preparations for six hours in the Soxhlet's apparatus, using boiling alcohol. Check preparations were made which had not been extracted. We found in the extracted specimens that the nuclei of red corpuscles in frog's blood showed the grass green color, which indicates the presence of phosphorus, after being treated with the stain. With human blood the leucocyte nuclei showed the green color and the plate masses also stained a pale green. This helps to confirm our opinion that the plates contain nuclear matter (see page 58).

Fischer[181] states that pure methyl green used in acid solution gives a chemical combination with nuclear matter, making it the only true microchemical staining test. It is of utmost importance that the methyl green be pure. As the stain is usually obtained,

it contains methyl violet as an impurity. This may be removed from an aqueous solution by shaking with chloroform or from the powdered dye by shaking with amyl-alcohol. There was a marked difference in the staining properties of the pure and the commercial dye. We shall confine ourselves to a description of the results obtained with the pure dye. As with all other stains the method of fixation makes a considerable difference. Some of the best results have been obtained in using the methyl green on fresh specimens or when it was mixed with formaldehyde and used as a fixative and staining fluid at the same time. After fixing in osmic acid we found that all the elements of the blood took the stain. This was true when the methyl green was added to the osmic acid at the time of fixation, and was equally true after all traces of the osmic acid had been removed by washing with water, and it also held good after passing osmic preparations through graded alcohols before staining. With aldehyde fixation methyl green gave striking results. The dye may be combined with the aldehyde and the fresh specimen stained and fixed at once in this mixture; or the preparation may first be stained before or after washing. Either of these methods gave, in general, the same results, but quicker results and clearer, sharper definitive staining was obtained, when the methyl green was added to the aldehyde at the time of fixing. If the solution was neutral or alkaline the staining was diffuse and very similar to that obtained when osmic acid was used as the fixative. If, however, the mixture of methyl green and aldelyde be acidified then true microchemical reactions may be obtained between the dye and the nuclear matter

and no other parts of the histological elements of the blood take the stain. This method was first tried on frog's blood where the nucleus and the body of the corpuscle could be well contrasted. It was afterwards tried on mammalian blood and showed the presence of nuclear matter in small granules in the plates. To acidify the mixture we employed either formic or acetic acid. These were used in different strengths from mixtures containing 2 1/2% formic acid to .001% formic acid; and from 1% acetic acid down to where the fluid would barely give an acid reaction. It was found that the strength of acid did not affect the reaction with nuclear matter, but the red corpuscles were deprived of their haemoglobin more or less rapidly according to the larger or smaller percent of acid. This was true down to .025% of formic acid below which the red corpuscles were not decolorized. In the reactions above given the aldehyde solutions contained .75% Na Cl. The effect of the absence of Na Cl has been discussed on pages 35-36. Our observations by this method on the reactions of blood-plates with methyl green were quite extensive and so far as we know, are the only such on record.

DRUEBIN WEIGHABLE MASSES OF BLOOD-PLATES.

Druebin[182] working with a modification of Von Mosen's[183] method claimed that by centrifugalization of blood drawn into ammonium oxalate, he could get weighable masses of blood-plates. We were led to doubt that he was working on plates alone, by his statement that if the end of a frog's heart were cut open beneath the

surface of a drop of ammonium oxalate, one would find, provided the preparation thus made were quickly sealed, structures which could not be distinguished from mammalian blood-plates. We repeated his experiment and found highly refractive bodies closely resembling blood-plates. In fact, but for their action toward 1% acetic acid, we should have called them plates; but with the acid they dissolve completely which proves beyond the shadow of a doubt that they are not the same.

DIGESTION WITH ARTIFICIAL GASTRIC-JUICE.

Some of the most valuable information concerning the chemical constitution of the blood-plates has been obtained by digestion with artificial gastric juice. This method has been employed by Lilienfeld[184] and by Sacerdotti[185]. These observers found that after indigestion by this method, an indigestible residue was left which they claimed to be nucleo proteid. We have repeated and extended the experiments of these observers and have found the results of such digestion depended greatly on the method of fixation. After hardening in formaldehyde (2.5% and above), none of the elements of the blood digested, even though the aldehyde had been thoroughly removed by washing. This would appear to destroy the value of the digestion method for studying the chemical constituents of the different blood corpuscles. We therefore extended our observations to include the effect of formaldehyde hardening on substances which are known to be readily digestible by hydrochloric acid and pepsin under ordinary

circumstances. As the best example of this type of substances, we chose fibrin obtained from whipping bullock's blood and subsequently preserved in 95% alcohol. Separate specimens of this were treated with different strengths of formaldehyde, then after thorough washing these were subjected to digestion with hydrochloric acid and pepsin under different conditions of pepsin concentration and of temperature. Side by side with these, control tests were made with the same fibrin which had not been subjected to the action of formaldehyde. The result of these experiments prove conclusively that formaldehyde has a profound effect on the digestibility by hydrochloric acid and pepsin of substances hardened in it. Therefore formaldehyde cannot be used as a fixative for blood-preparations in this line of work. The results of our experiments with fibrin are given in the following table.

Time in Aldehyde	Pepsin Solution	Temperature	Aldehyde Solution	Time of digestion
12 hrs.	10%	18°C	5%	Not digested in 3 days
12 hrs.	10%	37°C	5%	Not digested in 3 days
12 hrs.	10%	18°C	2.5%	At end of 12 hrs.
12 hrs.	10%	37°C	2.5%	At end of 12 hrs.
12 hrs.	10%	18°C	1%	At end of 12 hrs.
12 hrs.	10%	18°C	.5%	At end of 12 hrs.
12 hrs.	10%	18°C	.1%	At end of 12 hrs.
12 hrs.	1%	18°C	5%	Not digested in 48 hrs.
12 hrs.	1%	37°C	5%	Not digested in 48 hrs.
12 hrs.	1%	18°C	2.5%	Not digested in 48 hrs.
12 hrs.	1%	18°C	1%	Not digested in 48 hrs.
12 hrs.	1%	18°C	.1%	Digested in 1 1/2 hrs.

As will be seen from our table, the strength of aldehyde as well as the strength of pepsin affects the time of digestion, the 10% pepsin working more rapidly than the 1%, and the stronger aldehyde solutions retarding the action longer than the weak.

We also tried the effect of osmic acid upon the fibrin specimens in the same way and we found that for a 1% pepsin solution the osmic acid, when not well washed out, retarded the action so much that the fibrin was undigested at the end of a week. With blood preparations hardened in 1/2% osmic acid for 30 minutes and well washed in .75% Na Cl before putting into the 10% pepsin solution, the reds digested at once leaving nothing but the leucocyte nuclei and the plate-granules. We decided that 1/2% osmic acid is the best preserving fluid for digestion experiments. In every case there was a granular residue left from the plates. It seemed to us that since we knew that the blood-plates contained nuclear matter, this residue might prove to be composed of nucleo proteid as has been suggested. Since methyl-green is the only staining test for nuclear matter, we thought it might be possible to stain the preparation with methyl-green before subjecting it to the influence of the artificial gastric juice. We found that this would not work because, although the stain was very good for preparations hardened in weak aldehyde, it was decolorized by the acid pepsin. With osmic preparations the methyl-green no

longer acted simply as a pure nuclear stain. After digestion of preparations hardened in weak aldehyde we found that this residue stained like nuclear matter with acid methyl-green.

Work on Bone Marrow.

It is generally accepted today that the red bone marrow is the blood-forming organ during adult life. In embryonic life the liver and the spleen take an active part in the formation of blood corpuscles but most observers agree that it is only in exceptional cases, such as after excessive haemorrhage or severe anaemia that these organs play any role in the formation of the blood elements during adult life. The origin of the plates, as we have before mentioned, has long been a disputed question. We believed that a study of the bond-marrow of young kittens after severe haemorrhage, when the blood would be rapidly regenerated would show the formation of plates either as independent elements, or from the red corpuscles or in successive stages culminating in the adult erythrocyte. We examined the marrow and were unable to see plates being formed, in fact, we found no plates at all in bone-marrow except in specimens which were taken wet and in which there was great opportunity for the plates to come from the blood or the lymph. Aschoff[186] states that there are few scattered plates in the bone-marrow. We have observed nucleated erythrocytes in great numbers in the marrow, and have seen the nuclei either in the process of extrusion or fragmentation, but nowhere have we seen undoubted

formation of the plates.

In our work on bone-marrow we followed in a general way along the lines laid down by Howell[187] and extended his operations by using purified methyl-green (He used the ordinary Grübler stain which has been shown to contain methyl-violet). We also used the methyl-green in acid solution which we had found in previous work on blood preparations to be the only true nuclear stains. While this gave us the most distinctive micro-chemical test for nuclear matter, the most striking contrast stain for bone-marrow was Delafield's haematoxylin and eosin. We also tried Muir's[188] methlyene-blue-fuchsin but the results were less satisfactory than with either of the above methods. One advantage of the haematoxylin and eosin method is that it differentiates the nuclear matter and detects the presence of haemoglobin at the same time. In addition to the work on the bone-marrow of kittens, we examined the blood using in each case one kitten from the litter as a control at the beginning and another which was left unbled as a control at the end of the experiment. The kittens were nursed by the mother cat and were as nearly as possible under the same conditions.

We found an increase in the number of unclenated red corpuscles after the hemorrhage but nothing was shown bearing on the origin or function of the blood-plates.

SUMMARY.

1. After repeating the experiments of eleven different observers, all of whom claimed to have obtained distinctive reactions for blood-plates with staining fluids, we agree with Fischer in concluding that methyl green is the only anline dye which gives a true microchemical reaction with any definite constituent of the blood-plates.

2. From the results of our experiments with methyl green and with artificial digestion with pepsin and hydrochloric acid, we believe that the blood-plates contain nucleoproteid.

3. From our experiments with stains supposed to be distinctive tests for haemoglobin, we find that these stains vary with the fixing reagent, but in no case have we found the blood-plates giving the haemoglobin reaction.

4. From a general survey of all our work on the chemical composition of blood-plates, we are unable to identify them, beyond doubt, as connected with either the leucocytes or the red corpuscles.

5. After repeating Arnold's experiments and finding the structures described by him as budding off from the erythrocytes, we must regard these structures as artefacts. They differ from true blood-plates in dissolving completely with pepsin and hydrochloric acid.

6. The results of our experiments in repeating Drueben's work on frog's blood show that we cannot regard the structures described by him, as blood-plates, since they dissolve in 1% acetic acid.

7. By the process of exclusion, on the evidence given in the above paragraphs, we must regard the plates at present as independent elements of the blood.

8. We admit the possibility that the blood-plates may develop into red corpuscles. We base this conclusion on the form of the blood-plates observed in animals after haemorrhage or after fractional defibrination. In a few instances we have seen structures which might possibly be taken as the intermediate forms described by Hayem, although we have never found them in such quantities as described by that author, or undoubtedly containing haemoglobin.

9. From their richness in nucleoproteid we are prepared to admit also the possibility of Afanassiew's theory that the blood-plates may become nucleated erythroblasts in the bone-marrow and thus make red blood corpuscles in this indirect way. We must look upon this theory at present as far from proved.

10. From our own experiments by the method of fractional defibrination, we are prepared to assert positively that the blood-plates are the only morphological element of the blood which disappears regularly and progressively during coagulation. We feel that we can claim for these observations that they furnish new proof which is as definite as anything heretofore published in connection with this subject. After fractional defibrination the number of red blood corpuscles falls for several days. In studying their return to normal, and comparing this return with the regeneration of the blood-plates, we have sometimes found what might be taken for a "crise hematoblastique" as described by Hayem, but this was not invariably present.

1. Donné. Comptes Rendus de l' Acad.des Sciences, Vol. XIV, pp.366-368; also Donne', Cours de Microscopic, etc., Paris 1844, p. 85, and Atlas, plate 6, fig. 21.

2. Zimmerman, Rust's Magazine f.d. gesammt, Heilkunde, 1846-8, Vol. LXVI, also Virch. Arch., 1860, Vol. XVIII, pp. 221-242.

3. Beale, Trans. Royal. Mic. Society, 1864, pp. 32 and 47.

4. Schultze, Arch.f. Mik. Anat., 1865, Vol. I, pp. 1-43.

5. Riess, Arch. f. Anat. u. Physiol., 1872, pp. 237-248.

6. Ranvier, Gaz. Med. de Paris, 1873, pp. 93-4; also Traité technique, Paris 1876-1882, p. 215.

7. Vulpian, Gaz. Med. de Paris, 1873, p. 94.

8. Nedevetzke, Cent. f. d. Med. Wiss., 1873, pp. 147-150.

9. Osler, Proceedings of the Royal Society London, 1874, pp. 391-398; also Monthly Miscroscop. Journ., 1874, pp. 141-148.

10. Schmidt, Pflüger's Arch. 1875, Vol. II, pp. 291-369 and 515-577.

11. Semmer, Inaug. Dissertation, Dorpat, 1874.

12. Boettscher, Virch.Arch., 1877, Vol. LXIX, p. 298.

13. Norris, Trans. Birmingham Philosoph. Society, 1879; also Lancet, 1882, Vol. I, p. 163.

14. Fusari, Arch. per le scienze mediche, 1886, Vol. X,p.246.

15. Mosso, Virch.Arch., 1887, Vol. CIX, p. 205.

16. Klebs, Ziegler's Beiträge, 1888, Vol. III, p. 124.

17. Welti, Ziegler's Beiträge, 1889, Vol. IV, p. 548.

18. Salvioli, Virch. Arch., 1891, Vol. CXXV, p. 365.

19. Bremer, Cent. f. d. med. Wiss, 1894, p. 338.

20. Wlassow, Beitr. zur path. Anat., 1894, Vol. XV, pp. 559-566.

21. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, p. 208.

22. Scherer, Zeitsch. f. Heilkunde, 1896, Vol. XVII, p. 66.

23. Sacerdotti, Archivo per le scienze mediche, 1893, Vol. XVII, p. 40.

24. Gibson, Journ. of Anat. and Physiol., 1886, p. 200.

25. Arnold, Virch. Arch., 1896, Vol. CXLV, p. 1.

26. Löwit, Sitzenber. der Wien Acad., 1887, Vol. XCV. Abth III

27. Foa, Ziegler's Beiträge, 1889, Vol. V.

28. Howell, Journ. of Morphol., 1890, Vol. IV, pp. 57-128.

29. Maraglino and Castellino, Zeitsch. f. klin. Med., 1892, Vol. XXI, p. 415.

30. Lavdowsky, Zeitsch. f. wiss. Mik., 1893, Vol. X, p. 4.

31. Wlassow, Beitr. zur path. Anat., 1894, Vol. XV, p. 568.

32. Maximow, Arch. f. Anat. u. Entwickelungsgeschichte, 1899, pp. 33-82.

33. Müller, Ztg. Beitr., Vol. XXIII, pp. 498-528.

34. Determann, Deutsch. Arch. f. klin. Med., 1898, Vol. LXI, pp. 365-411.

35. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, p. 206.

36. Maximow, Arch. f. Anat. und Entwickelungsgeschichte, 1899, p. 47.

37. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, p. 207.

38. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, p. 205.

39. Engel, Arch. f. mik. Anat., 1893, Vol. XLII, p. 217.

40. Howell, Journ. of Physiol., 1891, Vol. IV, p. 106.

41. Sacerdotti, Archivo per le scienze mediche, 1893, Vol. XVIII, p. 40.

42. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, p. 207.

43. Riess, Arch. f. Anat. u. Physiol., 1872, p. 207.

44. Riess, Berliner klin. Wochenschr., 1879, p. 696.

45. Halla, Zeitschr. f. Heilkunde, 1883, pp. 376-378.

46. Pouchet, Gaz. Méd. de Paris, 1878, p. 136.

47. Howlet, Lancet, 1882, Vol. I, pp. 223-224.

48. Bizzozero, Virch. Arch., 1882, Vol. XC, pp. 261-332.

49. Hlava, Arch. f. exp. Path. and Pharm., 1883, Vol. XVII, pp. 403-409.

50. Kemp, Studies from the Biol. Lab. Johns Hopkins University, 1886, Vol. III, pp. 314-315.

51. Prus, Referat in Cent. f. klin. Med., 1887, Vol. VIII, p. 469.

52. Pizzini, Reforma Medica, 1894, Vol. II, pp. 388-391.

53. Rabl, Wien klin. Wochenschr., 1896, Vol. IX, p. 1061.

54. Van Emden, Fortsch. d. Med., 1898, Vol. XVI, p. 294.

55. Hirschfeld, Virch. Arch., 1901, Vol. CXLVI, p. 203.

56. Lilienfeld, Arch. f. Anat. u. Physiol., 1892, pp. 142-145.

57. Howell, Journ. of Morphol., 1890, Vol. IV, pp. 109-111.

58. Bremer, Cent. f. d. med. Wiss., 1894, p. 339.

59. Lavdowsky, Zeitschr. f. wiss. Mik., 1893, Vol. X, p. 21.

60. Schultze, Arch. f. mik. Anat., 1865, Vol. I, p. 36.

61. Osler, Monthly Microscopical Journ., London, 1874, p.144.

62. Osler, Cent. f. d. med. Wiss., 1882, p. 530.

63. Bizzozero, Cent. f. d. med. Wiss., 1882, p. 17.

64. Bizzozero, Arch. Ital. de Biol., 1882, Vol. II, p. 348.

65. Laker, Sitzungberichte d. kaiserlichen Acad. d. Wiss., Wien, 1883, Vol. LXXXVI, p. 193. Abth III

66. Lavdowsky, Synopsis in Hoffman and Schwalbe's Jahresbericht, Vol. XII, p. 64.

67. Halla, Zeitschr. f. Heilkunde, 1883, p. 378.

68. Schimmelbusch, Fortschr. d. Med., 1885, p. 100.

69. Schimmelbusch, Virch. Arch., 1885, Vol. CI, p. 227.

70. Petrone, Gazetta degli ospedali etc., 1897, p. 934.

71. Kemp, Studies from the Biol. Lab., Johns Hopkins University, 1886, Vol. III, p. 319.

72. Löwit, Virch. Arch., 1889, Vol. CXVII, p. 566.

73. Löwit, Sitzenber. d. kais. Acad., 1884, Vol. XC, p. 89. abth. III

74. Woolridge, Gerinnung des Blutes, Leipzig, 1891.

75. Bizzozero, Arch. Ital. de Biol., 1891, Vol. XVI, p.376.

76. Bizzozero, Cent. f. d. med. Wiss., 1882, p. 17.

77. Lavdowsky, Synopsis in Hoffman and Schwalbe's Jahresbericht, Vol. XII, p. 64.

78. Hlava, Arch. f. exp. Path. u. Pharm., 1883, Vol. XVII, p. 403.

79. Schimmelbusch, Fortschr. d. Med., 1885, p. 100.

80. Osler, Proceedings of the Royal Society, London, 1874, No. 153, pp. 391-398; and Cent. f. d. med. Wiss., 1882, pp. 529-531.

81. Pouchet, Gaz. Med. de Paris, 1878, p. 136

82. Bizzozero, Arch. Ital. de Biol., 1891, Vol. XVI, pp. 377-378.

83. Laker, Virch. Arch., Vol. CXVI, p. 18.

84. Sacerdotti, Archivo per le scienze mediche, 1893, Vol. XVII, pp. 43-44.

85. Löwit, Virch. Arch. 1889, Vol. CXVII, p. 566.

86. Lavdowsky, Zeitschr. f. wiss. Mik., 1893, Vol. X, p.21.

87. Wlassow, Beitr. z. Path. Anat., 1894, Vol. XV, pp. 559-566.

88. Arnold, Virch. Arch., 1896, Vol. CXLV, p. 1.

89. Bettmann, Ziegler's Beitr., 1898, Vol. XXIII, p. 420.

90. Hirschfeld, Virch. Arch., 1901, Vol. CXLVI, p. 206.

91. Determann, Deutsch. Arch. f. klin. Med., 1898, Vol. LXI, pp. 365-411.

92. Hayem, Du Sang, Paris, 1889

93. Hayem, Gaz. Med. 1880, p. 120.

94. Reyne, Thèse de Paris, 1881

95. Riess, Berlin. klin. Wocheuschr., 1879, p. 696.

96. Ehrlich, Berlin. klin. Wochenschr., 1881, p. 43.

97. Obrastzoff, Virch. Arch., 1881, Vol. LXXXVIII, pp. 408-410.

98. Afanassiew, Deutsch. Arch. f. klin. Med., 1884, Vol. XXXV, p. 250.

99. Druebin, Inang. Dissertation, Jurjew, 1893, p. 39.

100. Leube, Berlin. klin. Wochenschr., 1879, p. 653.

101. Neuman, Zeitschr. f. klin. Med., 1881, p. 434.

102. Hayem, Du Sang, Paris, 1878, pp. 286-287.

103. Kemp, Studies in the Biology Lab., Johns Hopkins University, 1886, Vol. III, p. 325.

104. Hayem, Arch. de Physiol., 1878, pp. 704-705.

105. Bizzozero, Arch. ital. de Biol., 1883, Vol. III, pp. 101-103.

106. Schultze, Arch. f. mik. Anat., 1865, Vol. I, pp. 36-37.

107. Osler, Cent. f. d. med. Wiss., 1882, p. 530.

108. Hayem, Arch. de Physiol., 1878, pp. 675,704.

109. Hayem, Gaz. Med., 1880, p. 215.

110. Leube, Berlin. klin. Wocheuschr., 1879, p. 654.

111. Bizzozero, Arch. Ital. de Biol., 1882, Vol. II, p. 352.

112. Bizzozero, Cent. f. d. med. Wiss., 1882, p. 18.

113. Davidson, Lancet, 1882, Vol. I, p. 1028.

114. Laker, Sitzungsher. d. kais. Akad., Wien, 1883, Vol. LXXXVI, p. 183. Abth. III.

115. Halla, Zeitschr. f. Heilkunde, Prag, 1883, p. 217.

116. Löwit, Sitzungsber. d. kais. Akad., Wien, 1884, Vol. LXXXIX, p. 297. Abth. III

117. Schimmelbusch, Fortschr. d. Med., 1885, p. 101.

118. Schimmelbusch, Virch. Arch., 1885, Vol. CI, p. 220.

119. Kemp, Studies from the Biological Lab., Johns Hopkins University, 1886, Vol. III, p. 321.

120. Mayer. Arch. de Physiol., 1878, pp. 675,704.

121. Bizzozero, Arch. ital. de Biol., 1882, Vol. II, p.352.

122. Kemp, Studies from the Biological Lab., Johns Hopkins University, 1886, Vol. III, p. 322.

123. Fusari, Archivo per le scienze mediche, 1886, Vol. X, p. 246.

124. Bizzozero, Arch. f. path. Anat., 1882, Vol. XC, p.301.

125. Laker, Sitzungsber. d. Kais. Akad., 1886, pp. 25-27. Abth III

126. Prus, Referat in Cent. f. klin. Med., 1887, Vol. VIII, p. 469.

127. Neumann, Zeitschr. f. klin. Med., 1881, p. 439.

128. Kemp, Studies in the Biological Lab., Johns Hopkins University, 1886, Vol. III, p. 322.

129. Lilienfeld, Arch. f. Anat. u. Physiol., Physiol. Abtheilung, 1892, p. 121.

130. Cavazzini, Archivo per le scienze mediche, 1893, Vol. XVII, p. 73.

131. Tschistowitsch, Cent. f. d. med. Wiss., 1894, pp. 242-243.

132. Muir, Journ. of Anat. and Physiol., 1890-1891, Vol. XXV, p. 267.

133. Petrone, Gazetta degli ospedali etc., 1897, p. 928.

134. Lilienfeld, Arch. f. Anat. u. Physiol., Physiol. Abth., 1892, pp. 147, 167-168.

135. Bizzozero, Arch. ital. de Biol., 1891, Vol. XVI, p.386.

135a. Kemp and Calhoun, Am. Journ. of Physiol., 1901, Vol. V, p. 4.

136. Laker, Sitzungsber. d. kais. Akad., 1886, Vol. XCIII, p. 32.

137. Kemp, Studies from the Biological Lab., Johns Hopkins University, 1886, Vol. III, p. 308.

138. Van Emden, Fortsch. d. Med., 1898, Vol. XVI, pp. 241-251.

139. Bizzozero, Zeitschr. f. wiss. Mik., 1892, Vol. IX, p. 231.

140. Hayem, Zeitschr. f. wiss. Mik., 1889, Vol. VI, p.375.

141. Brodie and Russell, Journ. of Physiol., 1897, Vol. XXI, p. 390.

142. Prus, Referat in Cent. f. klin. Med., 1887, p. 469.

143. Petrone, Cent. f. innere Med., 1895, p. 633.

143a. Marcano, Arch. de Med. Exper., 1899, Vol. XI, p. 434.

144. Daland, Fortschr. d. Med., 1891, Vol. IX, p. 823.

145. Arthus, C. R. Soc. de Biol., 1902, p. 214; also Journ. de Physiol. et Pathol. , 1902, Vol. IV, p. 273.

146. Fischer, Fixirung, Farbung und Bau des Protoplasmas, Jena, 1899.

147. Marcano, Arch. de Méd. Exper., 1899, Vol. XI, pp. 434-441; also Zeitschr. f. wiss. Mik., 1899, Vol. XVI, p. 364.

148. Japha, Deutsch. med. Wochenschr., 1901, Vol. XXVII, p. 334.

149. Marcano, Zeitschr. f. wiss. Mik., 1899, Vol. XVI, p. 364.

150. Benario, Quoted from Marcano's paper in Zeitschr. f. wiss. Mik., 1899, Vol. XVI, p. 364.

151. Marcano, Zeitschr. f. wiss. Mik., 1899, Vol. XVI, p. 364.

152. Lilienfeld, Arch. f. Anat. u. Physiol., 1892, pp. 147, 167-168.

153. Macallum, Trans. of the Canadian Institute, 1893, No. 4, Vol. II, p. 324.

154. Macallum, Proc.of. Roy. Mic. Soc., Vol. LXIII, p. 470.

155. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, pp. 199-200.

156. Michaelis and Wolff, Virch. Arch., 1902, Vol. CLXVII, p. 753.

157. Japha, Deutsch. med. Wochenschr., 1901, Vol. XXVII, p. 224.

158. Willebrand, Deutsch. med. Wochenschr., 1901, Vol. XXVII, pp. 57-58.

159. Becker, Deutsch. med. Wochenschr., 1901, Vol. XXVII, p. 78.

160. Engel, Deutsch. med. Wochenschr., 1901, Vol. XXVII, p. 323.

161. Bremer, Centr. f. d. med. Wiss., 1894, p. 339.

162. Jenner, Journ.of Roy Mic. Soc., 1899, p. 271.

163. Maximow, Arch. f. Anat., 1899, p. 39.

164. Dominici,

165. Hirschfeld, Virch. Arch., 1901, Vol. CLXVI, pp. 199-200.

166. Michaelis and Wolff, Virch. Arch., 1902, Vol. CLXVII, p. 153.

167. Romanowsky, Zeitschr. f. Hygiene, 1899, Vol. XXX, p.1.

168. Japha, Deutsch. med. Wochenschr, 1901, Vol. XXVII, p. 224.

169. Willebrand, Deutsch. med. Wochenschr, 1901, Vol. XXVII pp. 57-58.

170. Engel, Deutsch. med. Wochenschr, 1901, Vol. XXVII, p. 223.

172. Bremer, Cent. f. d. med. Wiss., 1894, p. 339.

173. Jenner, Journ. of Roy. Mic. Soc., 1899, p. 231.

174. Maximow, Arch. f. Anat., 1899, p. 39.

175. Dominici,

176. Muir, Journ. of Anat. and Physiol., 1890-1891, Vol. XXV, 258.

177. Rabl, Wien. klin. Wochenschr., 1896, Vol. IX, p. 1060.

178. Macallum, Trans. of the Canadian Institute, 1892, No. 4, Vol. II, pp. 221-260.

179. Erlicki, Warschauer med. Zeit., Vol. XXII, Nos. 15 and 18,

180. Macallum, Proc. of Roy. Mic. Soc., London, Vol. 67, p. 470.

181. Fischer, Fixirung, Farbung und Bau des Protoplasmas Jena, 1899.

182. Druebin, Inaugural Diss., Jurjew, 1893, pp. 1-18.

183. Von Mosen, Arch. f. Physiol., 1893, p. 353.

184. Lilienfeld, Arch. f. Anat. u. Physiol., 1892, pp. 142-145.

185. Sacerdotti, Archivo per le scienze mediche, 1893, Vol. XVII, p. 40.

186. Aschoff, Virch. Arch., 1892, Vol. CXXX, p. 105.

187. Howell, Journ. of Morphol., 1890, Vol. IV, pp. 117-130.

188. Muir, Journ. of Anat. and Physiol., 1890-1891, Vol. XXV, p. 267.

Afanassiew,- Ueber den dritten Formbestandtheil im normalen und pathologischen Zustande, und über die Beziehung desselben zur Regeneration des Blutes.- Deutsches Archiv für klinische Medicin, 1884, Vol. XXXIII, p. 217.

Arnold, Fr.- Anatomie, Vol. I, p. 181.

Arnold, J.- Ueber die Geschicke der leukocyten bei der Fremd-körperemboli.- Virchow's Archiv, 1893, Vol. CXXXIII, pp. 1-17.

Arnold, J.- Zur Morphologie und Biologie der Zellen des Knochenmarkes.- Virchow's Archiv, Vol. CXL, pp. 411-448.

Arnold, J.- Ueber die feinere Structur der haemoglobinlosen und haemoglobinhaltigen Knochenmarkzellen.- Virchow's Archiv, Vol. CXLIV, pp. 67-85.

Arnold, J.- Zur Technik der Blutuntersuchung.- Centralblatt für allgemeine Pathologie und pathologische Anatomie, 1896, Vol. VII, pp. 705-708.- Review of the same in Vol. VIII, 1897. pp. 294-295.

Arnold, J.- Zur Morphologie und Biologie der rothen Blutkörper.- Virchow's Archiv, 1896, Vol. CXLV, pp. 1-29.

Arnold, J.- Die korpusculären Gebilde des Froschblutes etc.- Virchow's Archiv, Vol. CXLVIII, pp. 470-500.

Arnold, J.- Zur Morphologie der extravasculären Gerinnung.- Virchow's Archiv, 1897, Vol. CL, pp. 444-470.

Arnold, J.- Ueber die Herkunft der Blutplättchen.- Centralblatt der allgemeine Pathologie und pathologische Anatomie, 1897, Vol. VIII, pp. 289-294.

Arnold, J.- Zur Morphologie intravasculären Gerinnung.- Virchow's Archiv, 1899, Vol. CLV, pp. 165-197.

Arthus, M.- Sur la vitesse etc.- Comptes Rendus des Séances de la Société de Biologie, 1902, p. 214.

Aschoff, Dr. L.- Ueber den Aufbau der menschlichen Thromben und das Vorkommen von Plättchen in den Blutbildenen Organen. Virchow's Archiv, 1892, Vol. CXXX, pp. 93-145.

Beale.- Observations on the nature of the red blood corpuscle.- Transactions of the Royal Microscopical Society, 1864, pp. 32-47.

Becker, E.- Ueber den Zusatz von Essigsäure zur Eosin.= Methylenblaulosung bei Färbung von Blutpräparaten.- Deutsche medicinische Wochenschrift, 1901, Vol. XXVII, p. 78.

Benario.- Quoted from Marcano's paper in Zeitschrift für wissen-shaftlichen Mikroscopie, 1899, Vol. XVI, p. 364.

Bettmann, Dr. S.- Ueber den Einfluss des Arseniks auf das Blut und das Knochenmark des Kaninchens.- Ziegler's Beiträge, 1898, Vol. XXIII, pp. 377-497.

Bizzozero.- Di un nuovo elemento morfologico del sangue dei mamiferi e della sua importanza nella trombosi e nella coagulazione.- R. Accademia di Medicina di Torino, 1881, pp. 124-128,- also,- Virchow's Archiv, 1882, Vol. XC, pp. 261-332,- also,- Archives Italiennes de Biologie, 1882, Vol. II, pp. 345-362, and Vol. III, 1883, pp. 94-121.

Bizzozero.- Sur les petites plaques du sang des mammiferes. Archives Italiennes de Biologie, 1882, Vol. I, pp. 274-278, also Centralblatt für die medicinischen Wissenschaften, 1882, pp. 17-20, and pp. 161-163.

Bizzozero.- Die Blutplättchen in peptonizirten Blut.- Centralblatt fur die medicinischen Wissenschaften, 1883, pp. 529-533.

Bizzozero and Sanquirico.- Sul destino dei globuli rossi nella transfusione di sangue defibrinato.- Archivo per le scienze mediche, 1886, pp. 341-353.

Boettscher.- Ueber einige Veränderungen welche die rothen Blutkörperchen in extravasaten erleiden.- Virchow's Archiv, 1877, Vol. LXIX, pp. 295-307 (With plate XI).

Blum, F.- Das Formaldehyd als Härtingsmittel.- Zeitschrift fur wissenschafttichen Mikroscopie, 1893, Vol. X, p. 314.

Bremer, Dr. L.- Ueber die Herkunft und Bedeutung der Blutplättchen.- Centralblatt für der medicinischen Wissenschaften.- 1894, p. 338.

Brodie and Russell.- The Enumeration of Bloodplatelets.- Journal of Physiology, 1897, Vol. XXI, pp. 390-395.

Cadet, A.- Étude Physiologique des Élements figurés du Sang et in particulier des Hématoblasts.- These de Paris, 1881.

Cavazzani, Dr. A.- Contrattilita delle emazie dei mammiferi. Archivo per le scienze mediche, 1893, Vol. XVII, pp. 57-74.

Daland.- Ueber das Volumen der rothen und weissen Blut-Körperchen im Blute des gesunden und Kranken Menschen. Fortschritte der Medicin, 1891, Vol. IX, pp. 823-833, 867-879.

Davidson.- A clinical study of the small granular cells of the blood.- Lancet, 1902, Vol. I, pp. 1022-1029.

Determann, Dr. H.- Klinische Untersuchungen über Blutplättchen.- Deutsches Archiv für klinische Medicine, 1898, Vol. LXI, pp. 365-411.

Dominici.-

Donné.- Comptes Rendus de l' Academie des Sciences, Paris, Vol. XIV, pp. 366-368.

Donné.- Cours de microscopie etc., Paris, 1844. (Atlas bound separately.)

Druebin, Dr. S.- Die Herstellung wägbarer Mengen von Blutplättchen bei Säugethieren und die wirklichen Blutplättchen des Frosches.- Inaugural Dissertation, Jurjew, 1893.

Erlich.- Ueber Regeneration und Degeneration der rothen Blutscheiben bei Anämien.- Berliner klinische Wochenschrift, 1880, p. 405,- also 1881, p. 43.

Engel, C. S.- Zur Enstehung körperlichen Elemente des Blutes.- Archiv für mikroskopische Anatomie, 1893, Vol. XLII, pp. 217-247.

Engel, C. S.- Zur Färbung von Blut- und Eiter-prapäraten mit Eosin-Methyleneblau.- Deutsche medicinische Wochenschrift, 1901, Vol. XXVII, p. 223.

Erlicki.- Warschauer medische Zeitschrift, Vol. XXII, Nos. 15, 18.

Fischer, G.- Fixirung, Farbung und Bau des Protoplasmas, Jena, 1899.

Foa.- Beitrag zum Studium der Structur der rothen Blutkörperchen der Säugethiere.- Ziegler's Beiträge, 1889, Vol. V.

Fusari, R.- Contributo allo studio delle piastrine del sangue allo stato normale e pathologico.- Archivo per le scienze mediche, 1886, Vol. X, pp. 235-274.

Gibson, L.- The Bloodforming Organs and Blood Formation; an Experimental Research.- Journal of Anatomy and Physiology, 1886, Vol. XX, pp. 324, 486, 674.

Gulland, L. G.- On the fixing and staining of blood films.- Zeitschrift für wissenschaftlichen Mikroscopie, 1897, Vol. XIV, p. 62, also, Vol. XXII, 1900, p. 220.

Halla.- Ueber den Haemoglobingehalt des Blutes, und die quantitativen Verhältnisse der rothen und weissen Blutkörperchen bei acuten fieberhaften Krankheiten.- Zeitschrift für Heilkunde, Prag, 1883, pp. 198-251, and 331-379.

Hayem.- Des caractères anatomiques du sang chez le nouveau né, pendant les premiers jours de la vie.- Sur la nature et la signification des petits globules rouges du sang. Comptes Rendus de l' Academie des Sciences, Paris, 1877, Vol. LXXXIV, pp. 1166-1169, 1239-1242.

Hayem.- Sur l' évolution des globules rouges dans le sang des vertébrés ovipares.- Sur l' evolution des globules rouges dans le sang des vertébrés vivipares.- Comptes Rendus de l' Académie des Sciences, Paris, 1877, Vol. LXXXV, pp. 907-909, 1285-1288.

Hayem.- Recherches sur l' anatomie normale et pathologique du sang.- Monograph, Paris, 1878.

Hayem.- Sur la formation de la fibrine du sang, étudiée au microscope.- Comptes Rendus de l' Académie des Sciences, 1878, Vol. LXXXVI, p. 58.

Hayem.- Note sur les caractères et l' évolution des hématoblastes chez les ovipares.- Gazette Médicale de Paris, 1878, pp. 15-17, 43-45, 49, 60-61, 107, 121-122, 257-258.330.

Hayem.- Recherches sur l' évolution des hématies dans le sang de l' homme et des vertébrés.- Archives de Physiologie, 1878, pp. 692-734;- 1879, pp. 201-261, 577-613.

Hayem.- Sur l' origin des hématoblastes.- Gazette Médicale de Paris, 1879, pp. 198, 565.

Hayem.- Sur la caracteres anatomique du sang.- Gazette Médicale de Paris, 1880, pp. 119-120, 191-192, 215-216.

Hayem.- Contribution à l' étude de la structure des hématoblastes et des hématies.- Gazette Médicale de Paris, 1881, pp. 479-481.

Hayem.- Contributions à l' étude des altérations morphologique des globules rouges.- Des globules rouges à noyau dan le sang de l' adulte.- Archives de Physiologie, 1883, Vol. I, pp. 214-223, 363-373.

Hayem.- Note sur les "Plaquettes du Sang" de M. Bizzozero, et sur la "Trosième Corpuscle du Sang" ou "Invisible Corpuscle" of M. Morris.- Gazette Médicale de Paris, 1883, pp. 432-433.

Hayem.- Expériences démonstrant que les concrétions sanguines formées au niveau d' un lesé des vaisseaux, déboutent par un dépôt d' hématoblastes.- Comptes Rendus de l' Académie des Sciences, 1883, Vol. XCVI, pp. 653-655.

Hayem.- Nouvelle contribution a l' étude des concrétions sanguines intravasculares.- Comptes Rendus de l' Académie des Sciences, 1883, Vol. XCVII, pp. 144-147.

Hayem.- De la crise hématique dans fièvre intermittente.- Archives de Physiologie, 1883, Vol. II, pp. 247-256.

Hayem.- Maladies du Sang, Paris, 1900.

Hirschfeld, Dr. H.- Ueber die Entstehung der Blutplättchen.- Archiv für pathologische Anatomie, 1901, Vol. CLXVI, pp. 195-211.

Hlava.- Die Beziehung der Blutplättchen Bizzozero's zur experimentelle Pathologie und Pharmakologie, 1883, Vol. XVII, pp. 392-418.

Hlava.- Zur Histogenese des Fibrins.- Fortschritte der Medicin, 1883, pp. 341-343.

Howell.- Life history of the formed elements of the blood; especially of the red corpuscles.- Observations upon the occurrence, structure and function of the giant cells of the marrow. Journal of Morphology, 1890, Vol. IV, pp. 57-130.

Howlet.- On the granular matter of the Blood.- Lancet, 1892, Vol. I, pp. 223-224.

Japha.- Zur Eosin-Methylenblaufärbung des Blutes.- Deutsche medicinische Wochenschrift, 1901, Vol. XXVII, p. 224.

Jenner, L.- New preparation for rapidly fixing and staining blood.- Journal of the Microscopical Society, 1899, p. 231; also Lancet, 1899, Vol. I, p. 370.

Kemp, G. T.- "New Element" of the Blood.- Studies from the Biological Laboratory, Johns Hopkins University, 1886, Vol. III, pp. 293-349.

Kemp and Calhoun.- Numeration of Blood-plates.- American Journal of Physiology, 1901, Vol. V, p. 4.

Klebs.-

Ziegler's Beiträge, 1888, Vol. III.

Laker.- Die Blutscheibchen sind constante Formelemente des normal circulirenden Säugethierblutes.- Virchow's Archiv, Vol. CXVI, pp. 28-49.

Laker.- Studien über die Blutscheibchen bei angeblichen Zerfall der weissen Blutscheibchen und den der Blutgerinnung.- Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Wien, 1883, Vol. LXXXVI, pp. 173-301. Abtheilung III

Laker.- Die ersten Gerinnungserscheinungen des Säugethier-Blutes unter dem Microscope.- Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Wien, Vol. XC, pp. 147-159. Abtheilung III

Laker.- Beobachtungen an den geformten Bestandtheilen des Blutes.- Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Wien, 1886, Vol. XCIII, pp. 25-27. Abtheilung III

Lavdowsky, Blut und Jodsäure und der sogenannte Chemotropismus.

Zeitschrift für wissenschaftlichen Mikroscopie, 1893, Vol. X, pp. 4-35.

Leube.- Ein Fall von essentieller Anämie mit übermässiger Entwickelung der Körnerbildungen um Blute.- Berliner Klinische Wochenschrift, 1879, pp. 653-655.

Lilienfeld, L.- Haematologische Untersuchungen.- Archiv für Physiologie, 1892, pp. 115-154.

Lilienfeld, L.- Ueber die Mikrochemische Localization des Phosphorors in den Geweben.- Archiv für Physiologie, 1892, pp. 548-550.

Lilienfeld, L.- Ueber den flüssigen Zustand des Blutes und die Blutgerinnung. Archiv für Physiologie, 1892, pp. 550-556.

Löwit.- Beiträge zür Lehre von der Blutgerinnung.- Sitzungsberichte der kaiserlichen Akademie, Wien, 1884, Vol. LXXXIX, pp. 270-307; continued in 1884, Vol. XC, pp. 80-132. abtheilung III

Löwit.- Die Blutplättchen und die Blutgerinnung.- Fortschritte der Medicin, 1885, pp. 173-178, 276-278.

Löwit.- Die Umwandlung der Erythroblasten in rothe Blutkörperchen.- Sitzungsbericht der kaiserlichen Akademie, Wien, 1887, Vol. XCV, pp. 129-179. abtheilung III

Löwit.- Beiträge zur Lehre von der Leukämie. Die Beschaffenheit der Leucocyten bei der Leukämie.

Sitzungsbericht der Kaiserlichen Akademie, Wien, III, 1887, Vol. XCV, pp. 227-245. abtheilung III

Löwit.- Ueber Blutplättchen und Thrombose.- Fortschritte der Medicin, 1888.

Löwit.- Ueber die Preexistenz der Blut Plättchen, und die Zahl der weissen Blutkörperchen im normalen Blutes des Menschen.- Virchow's Archiv, 1889, Vol. CXVII, p. 345.

Macallum.- On the detection and localization of phosphorus in animal and vegetable tissues.- Proceedings of the Royal Microscopical Society, Vol. LXIII, p. 470.

Macallum.- Studies on the blood of amphibia.- Transactions of the Canadian Institute, 1892, No. 4, Vol. II, pp. 321-260.

Maraglino and Castellino.- Ueber die langsame Nekrobiosis der rothen Blutkörperchen sowohl in normalem wie auch in pathologischem Zustande und ihren semiologischen und klinischen Werth.

Zeitschrift für klinische Medicin, 1892, Vol. XXI, pp. 415-453.

Marcano.- De l' action de formol sur les globules rouges du sang.- Archives de Médicine Expérimentale, 1899, Vol. XI, pp. 434-441; also Zeitschrift für wissenschaftlichen Mikroscopie, 1899, Vol. XVI, p. 364.

Matthes.- Weitere Beobachtungen über den Austritt des Haemoglobins aus sublimatgehärteten Blutkörperchen.- Münchiner medicinische Wochenschrift, 1902, Vol. XLIX, pp. 698-700.

Maximow, Dr. A.- Ueber die Struktur und Entkernung der rothen Blutkorperchen der Säugethiere und über die Herkunft des Blutplättchen.- Archiv für Anatomie und Entwicketungsgeschichte, 1899, pp. 33-82.

Michaelis, L.- Ueber die Methylenblau-Eosinfärbung.- Deutsche medicinische Wochenschrift, 1901, Vol. XXVII, p. 127.

Michaelis and Wolff.- Ueber Granula in Lymphocyten.- Virchow's Archiv, 1902, Vol. 167, pp. 151-160.

Mosen.- Herstellung wägbarer Mengen von Blutplättchen.- Archiv für Physiologie, 1893.

Mosso.- Die Umwandlung der rothen Blutkörperchen in Leukocyten und die Nekrobiose der rothen Blutkorperchen bei der Coagulation und Eiterung, Virchow's Archive, 1887, Vol. CIX, p. 205-377.

Muir.- Contributions to the Physiology and Pathology of the Blood.- Journal of Anatory and Physiology, 1890-1891, Vol. XXV, pp. 256-277, 352-374, 475-505.

Müller, H. F.- Zur Frage der Blutgerinnung.- Sitzungsberichte der kaiserlichen Akademie, Wien, 1889, Vol. XCVIII. Abtheilung III

Müller, H. F.- Die morphologischen Veränderungen der Blutkörperchen und des Fibrins bei der vitalen extravasculären Gerinnung.- Ziegler's Beiträge, Vol. VIII, pp. 491-524; abstracted in Centralblatt für allgemeine Pathologie und pathologische Anatomie, 1891, Vol. VIII, pp. 692-697.

Nedrygailoff.- Zur Histologie des Herzenblutes.- Centralblatt für der medicinischen Wissenschaften, 1873, pp. 147-150.

Neumann.- Ueber Blutregeneration und Blutbildung.- Zeitschrift für klinische Medicin, 1881, Vol. III, pp. 411-449.

Norris.- Transactions of the Birmingham Philosophical Society, 1879.

Norris.- The new blood corpuscle.- Lancet, 1882, Vol. I. p. 163.

Norris.- On the claim of Professor Bizzozero to the discovery of the fibrin-forming corpuscle.- Lancet, 1882, Vol. I, pp. 561-562.

Obstrastzoff.- Zur Morphologie der Blutbildung im Knochenmark der Säugethiere.- Virchow's Archiv, 1881, Vol. LXXXIV, pp. 358-414.

Osler.- An account of certain organisms occuring in the liquor sanguinis.- Proceedings of the Royal Society, London, 1874, No. 153, pp. 391-398.

Osler.- Ueber den dritten Formbestandtheil des Blutes.- Centralblatt für der medicinische Wissenschaften, 1882, pp. 529-531.

Petrone.-

Reforma Medica, 1891, Vol. XXXV.

Petrone.-

Gazetta degli ospidali etc., 1897, p. 934.

Pizzini, Dr. L.- Modificazione fisio-pathologische delle piastrine del sangue, specialmente in rapporto con gli aumenti di temperatura.- Reforma Medica, 1894, Vol. pp. 374-379, 386-391.

Pizzini and Fornaca.- Nota preventiva sulle modificazione delle piastrine nella febbre.- Reforma Medica, 1894, No. 62.

Pouchet.- Note sur la régénération des hematies.- Gazette Médicale de Paris, 1879; pp. 184-185, 209.

Prus.- Einiges über das Verhalten des leukämischen Blutes.- Referat in Centralblatt für klinische Medicin, 1887, p. 496. Original paper in Polish, Medycyna, 1886, Nos. 39, 40.

Rabl.- Ueber eine elective Färbung der Blutplättchen in Trockenpräparaten.- Wiener klinische Wochenschrift, 1896, Vol. IX, pp. 1060-1062.

Ranvier.- Traité technique d' Histologie, Paris, 1876-1882.

Ranvier.- Du mode de formation de la fibrine dans le sang extrait des vaisseaux.- Gazette Médicale de Paris, 1873, pp. 93-94.

Reyne.- De la crise hématique dans les maladies aiguës a déferoescence brusque.- Thèse de Paris, 1881.

Riess.- Zur pathologischen Anatomie des Blutes.- Archiv für Anatomie und Physiologie, 1872, pp. 837-848.

Riess.- Ueber sogenannten Micrococcen.- Centralblatt für der medicinische Wissenschaften, 1873, pp. 530-533.

Riess.- Bemerkungen über die Zerfallskörperchen des Blutes und ihr Verhältniss zur Anämie.- Berliner klinische Wochenschrift, 1879, pp. 696-697.

Romanowsky.- Zur Frage der Parasitologie und Therapie der Malaria.- Zeitschrift für Hygiene, 1899, Vol. XXX, p. 1.

Sacerdotti.- Intorno alle Piastrine del Sangue.- Archivo per le scienze mediche, 1887, Vol. XVII.

Salvioli, Dr. J.- Ueber die Todesursachen nach Verbrennung.- Virchow's Archiv. 1891, Vol. CXXV, p. 378.

Scherer.- Ueber Zoöid- und Oekoidbildung in den rothen Blutkörperchen und ihre Beziehung zur Thrombose.- Zeitschrift für Heilkunde, 1896, Vol. XVII, pp. 49-69.

Schirmelbusch.- Die Blutplättchen und die Blutgerinnung.- Fortschritte der Medicin, 1885, pp. 97-103; also Virchow's Archiv. 1885, Vol. CI, pp. 201-244.

Schmidt.- Ueber die Beziehung der Faserstoffgerinnung zu den körperlichen Elementen des Blutes.- Pflügers Archiv, 1875, Vol. XI, pp. 291-369, 515-577.

Schultze.- Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes.- Archiv für mikroskopische Anatomie, 1865, Vol. I, pp. 1-43.

Semmer.- Ueber die Faserstoffbildung im Amphibien- und Vogel- blut, und die Entstehung der rothen Blutkörperchen der Saügethiere.- Inaugural Dissertation, Dorpat, 1874.

Tschistowitsch.- Zur Frage über Leucocolyse. Centralblatt für der medicinische Wissenschaften, 1894, pp. 242-243.

Van Emden.- Klinische Untersuchungen über die Blutplättchen.- Fortschritte der Medicin, 1898, Vol. XVI, pp. 241-251, 282-295.

Vulpian.-

Gazette Médicale de Paris, 1873, p. 94; also Comptes Rendus des Séances de la Société de Biologie, 1873, p. 49.

Welti, Dr. E.- Ueber die Todtes Ursachen nach Hautverbrennung.- Ziegler's Beiträge, 1888, Vol. IV, pp. 519-567.

Wilbrand, H. A.- Eine Methode für gleichzeitige Conservationsfärbung von Bluttrockenpräparaten mit Eosin und Methylenblau.- Deutsche medicinische Wochenschrift, 1901, Vol. XXVII, p. 57.

Wlassow.- Untersuchungen über die histologischen Vorgänge bei der Gerinnung und Thrombose mit besondere Berucksichtigung der Entstehung der Blutplättchen.- Beiträge zur pathologische Anatomie, 1894, Vol. XV, pp. 547-580.

Wooldridge.- Gerinnung des Blutes.- Monograph, Leipsig, 1891.

Zimmerman.- Ueber die Formgebilde des menschlichen Blutes, in ihrem nähern Verhältniss zu Process der Entzundung und Eiterung.- Rust's Magazin für die gesamte Heilkunde, 1846-1847. Vol. LXVI.

Zimmerman.- Die Blutkörperchenfrage.- Virchow's Archiv, 1860, Vol. XVIII, pp. 221-242.

Explanation of Curves

Throughout the curves the number of red corpuscles is indicated by the red line.

The specific gravity is indicated by the black line.

The number of blood-plates is indicated by the green line.

The color of the figures at the left margin of the plate indicates the curve to which such figures belong.

The red figures,- to be read with red curve only,- give the number of red corpuscles in millions and hundred-thousands.

The black figures,- to be read with black curve only,- give the specific gravity directly.

The green figures,- to be read with green curve only,- give the number of blood-plates in thousands.

The figures in black at the lower margin of the plate have the same significance in all the curves, as follows:-

Number 1' shows the count made the day of the defibrination.

Number 2' shows the count made the day before the defibrination.

Number 3' shows the count made the day preceding number 2', etc.

Number 1 shows the count made after the first defibrination.

Number 2 shows the count made after the second defibrination.

Number 3 shows the count made after the third defibrination,- etc.

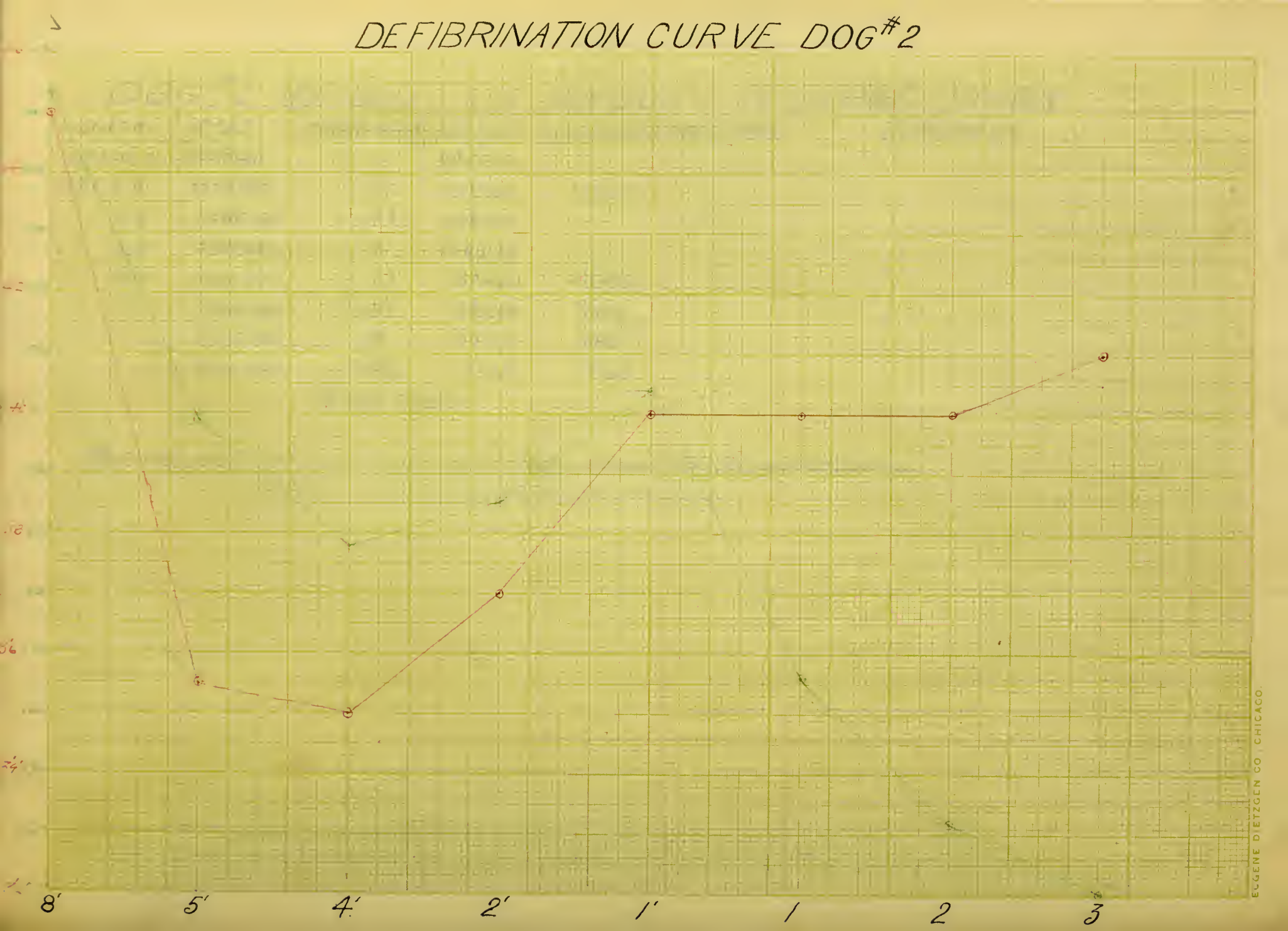
Number 1a shows the count made the first day after the defibrination.

Number 2a shows the count made the second day after the defibrination, etc.

DOG #1 WEIGHT — AMOUNT OF BLOOD DRAWN

DATE	REDS	PLATE:RED	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
APR. 10, 1901	4'600'000	1:10.1	460'000	10'000	----------	11 A.M. BEFORE DEFIBRINATION
" " 6 P.M.	3'750'000	---------	0 (?)	7'600	----------	AFTER DEFIBRINATION. MASS OF BROKEN DOWN PLATES
APR. 11, 1901	3'75'0'000	1:14.5	272'000	29'000	----------	
APR. 12, 1901	3'950'000	1:9.3	409'000	19'000	----------	
APR. 13. 1901	3'950'000	1:10.5	376'000	21'800	----------	DOG IN GOOD CONDITION
APR. 15, 1901	4'000'000	1:11.3	352'000	25'000	----------	NO FOOD FOR 60 HOURS
APR. 16, 1901	4'200'000	1:10.3	406'000	25'400	----------	
APR. 17, 1901	4'000'000	1:11.0	364'000	30'000	----------	BLOOD CLOTS RAPIDLY

DEFIBRINATION CURVE DOG #2

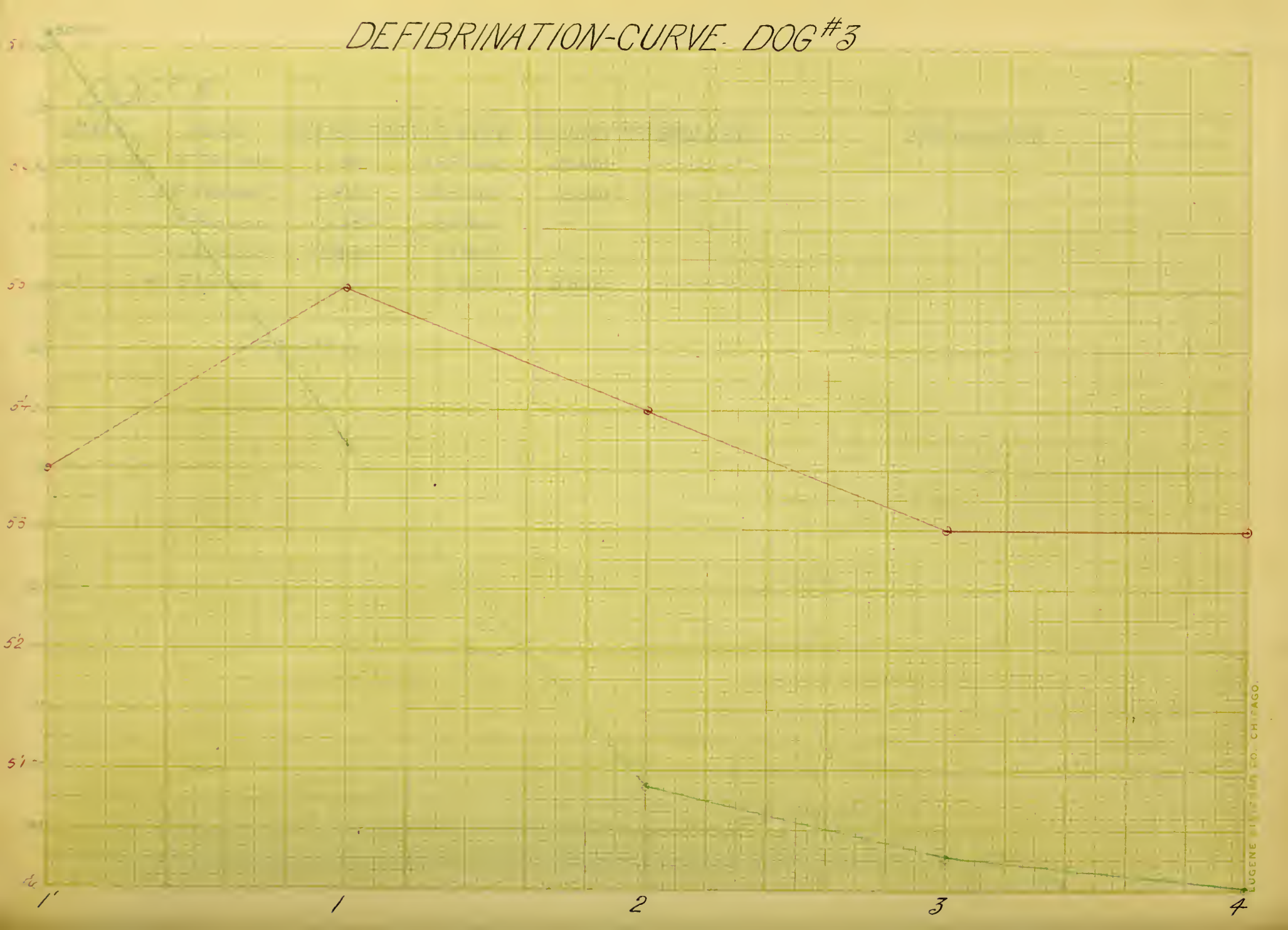


DOG #2 WEIGHT--- K AMOUNT OF BLOOD DRAWN

DATE	REDS	PLATES:REDS	PLATES	LEUCOCYTES	SPEC. GRAV	REMARKS
APR. 30. '01.	4'500'000	1:19	490'000	-------	--- ----	
MAY 3, '01.	3550'000	1:11.5	307'000	17'000	--------	
" 4, '01	3'580'000	1:14.3	244'000	------	-------	
" 6, 01	3700'000	1:14.	266'000	------	------	
" 8, 01	4000'000	1:12.5	322'000	22'800	-----	
" " " 1	4'000'000	1:22.4	178'000	18'000	-----	
" " " 2	4'000'000	1:38.	105'000	15'800	-----	
" " " 3	4'100'000	1:58.2	70'000	13'600.		
		DOG DIED.				

POST MORTEM. NO HEART CLOT. NO PEPTONE IN URINE. INTESTINES ULCERATED.
ANIMAL INFESTED WITH DOG-SUCKING LOUSE.

DEFIBRINATION-CURVE. DOG #3

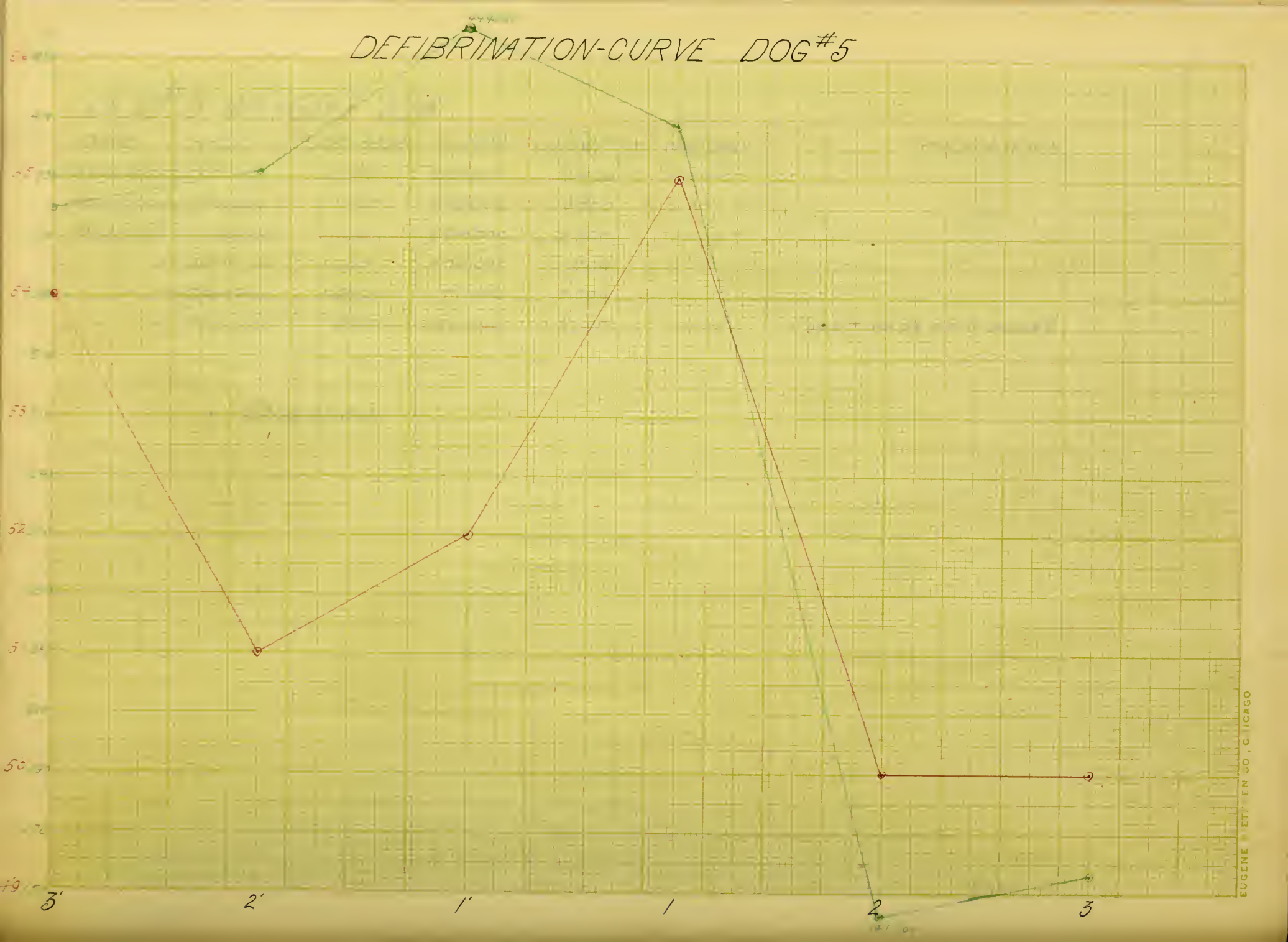


DOG #3

DATE	REDS	PLATES:REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
MAY 14. 1901	5'350'000	1:15.1	357'000	21'600	-------	
" " 1.	5'500'000	1:29.0	185'000	16'800	-------	
" " 2.	5'400'000	1:124.2	43'000.	------	-------	
" " 3.	5'300'000	1:384.	13'000	------	-------	
" " 4.	5'300'000.	-----	0(?)	8'600	-------	

DOG DIED

"D. G."

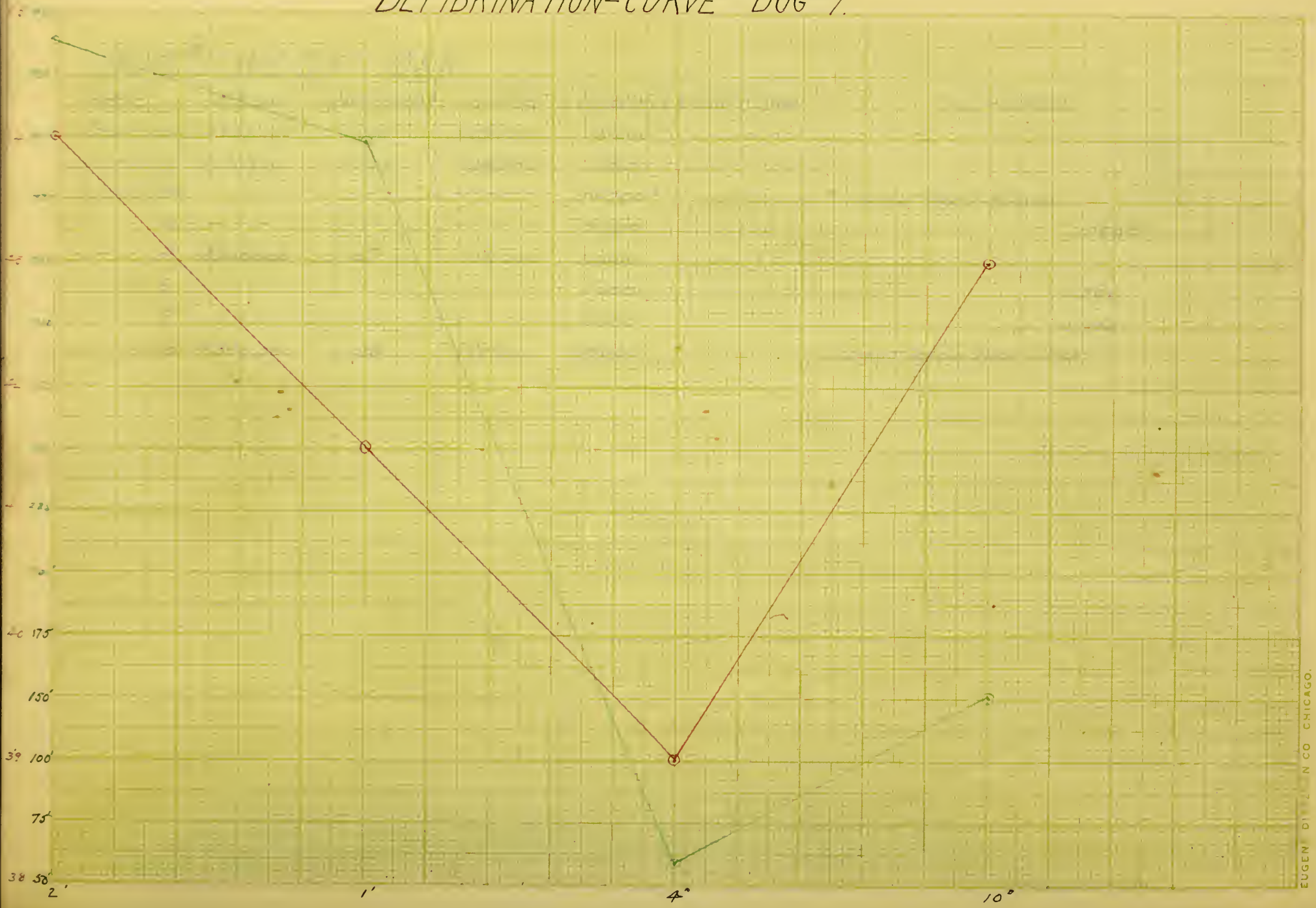

DEFIBRINATION-CURVE DOG #5
52
50
3'
2'
1'
1
2
3
EUGENE ETTENSON, CHICAGO

DOG #5 WEIGHT 10K.

DATE	REDS	PLATES : REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
MAY 26 1901	5'400'000	1:14.3	379'000	16'400	-------	
MAY 27, 1901	5'100'000	1:14.0	392'000	10'000	-------	
MAY 28 1901	5'200'000	1:11.6	444'000	8'800	------	
" " " 1:	5'500'000	1:13.5	408'000	5'200	-------	
" " " 3.	5'000'000	1:35.5	141'000	3'600	-------	
" " " 4.	5'000'000	1:32.0	156'000	9'600.	---	COUNT MADE FROM HEART.

DOG DIED

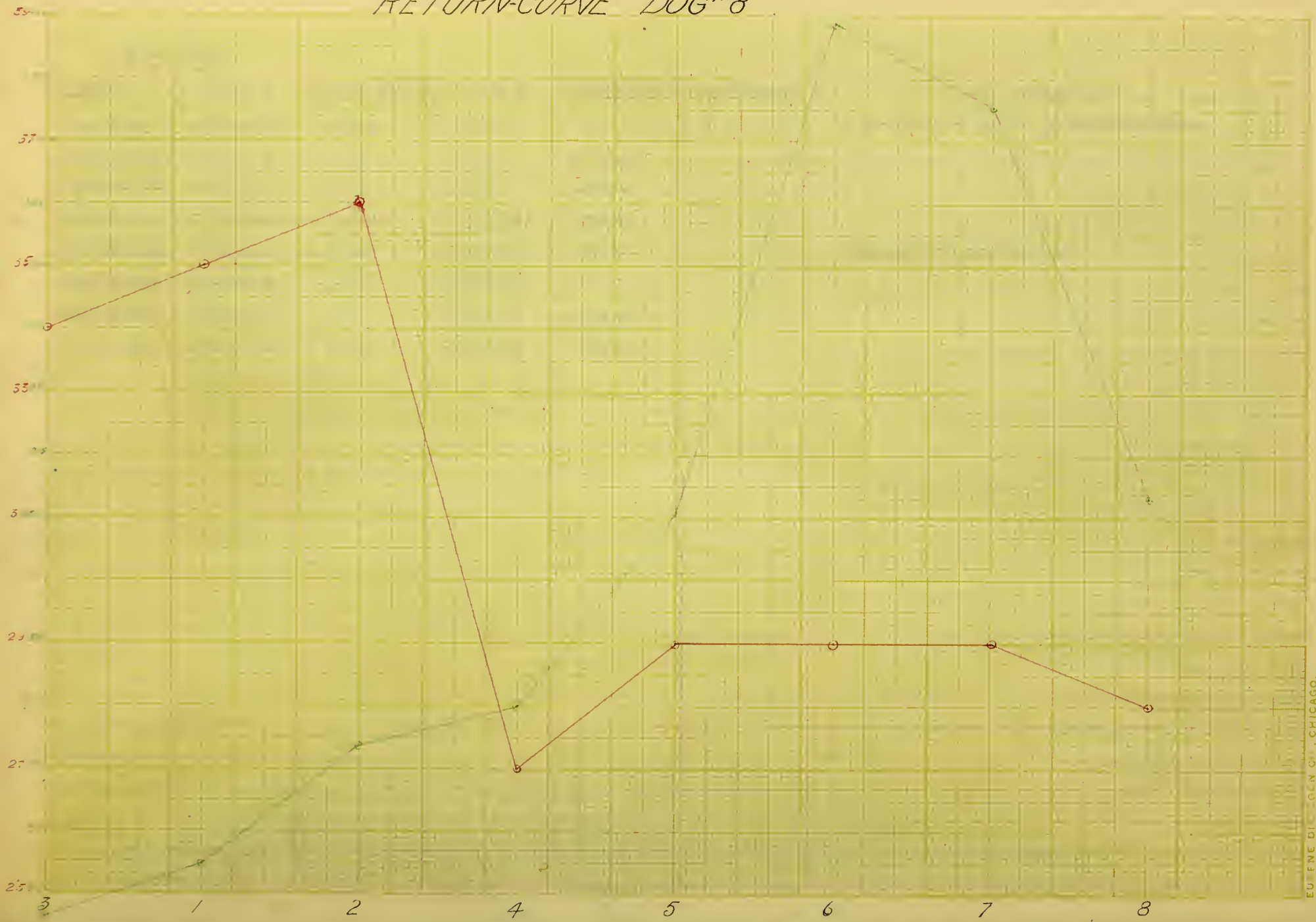
DEFIBRINATION-CURVE DOG #7.



DOG #7 WEIGHT 10.5K

DATE	REDS	PLATES:REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
JULY 13, 1901	4'400'000	1:10.1	439'000	15'200	-------	
JULY 15, 1901	4'150'000	1:10.4	399'000	15'800	-------	
JULY 15, 1901	-----	----	-----	14'200 *	-------	* IN FILTERED BLOOD
" " " 2.	-----	-----	-----	12'200	------	" " " " " " 10'800
" " " 4.	3'900'000	1:67.	58'000	11'000	------	
" " " 6.	------	-----	------	7'600	------	" " " " " " 6'200
" " " 9.	------	----	------	7'000	------	" " " " " " 6'600
" " " 10.	4'300'000	1:28	150'000	24'800	-------	COUNT MADE FROM HEART.

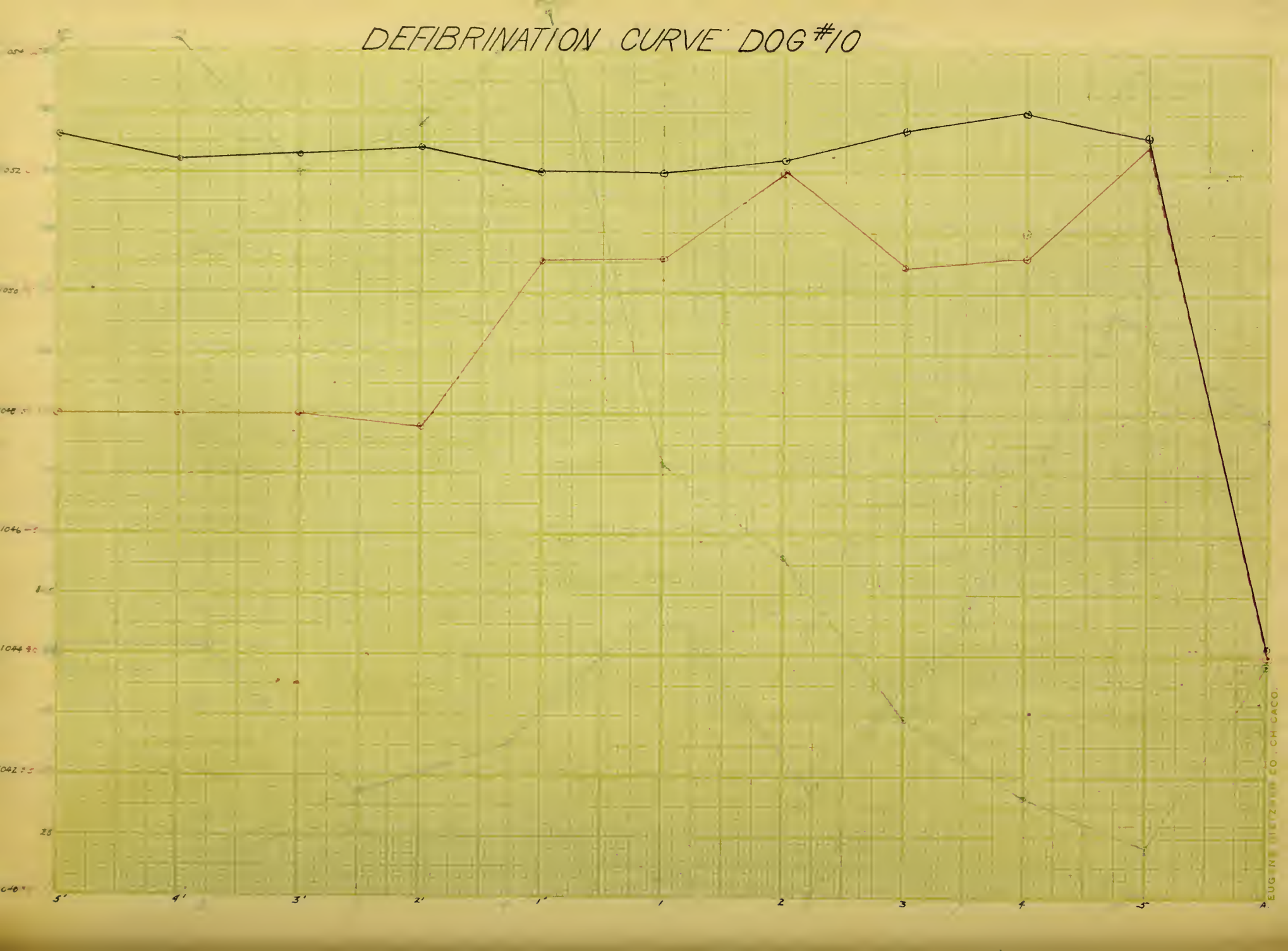
RETURN-CURVE DOG #8



DOG #8

DATE	REDS	PLATE:REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
JUNE 28 '01	3'400'000	1:216.	16'000	---------	--------	AFTER THE THIRD DEFIBRINATION
JUNE 29 01	3'500'000	1:95.	37'000	27'600	-------	
JUNE 30 1901	3'600'000	1:33.	109'000	13'400	-------	
JULY 2, 190:	2'700'000	1:21.6	125'000	18'400	-------	
JULY 3, 1901	2'900'000	1:11.5	252'000	19'200	-------	WOUND SUPPURATING
JULY 4, 1901	2'900'000	1:7.3	397'000	------	-------	
JULY 5, 1901	2'900'000	1:8	363'000	37'200	------	
JULY 6 1901	2'800'000	1:10.	280'000	35'200	-------	

DEFIBRINATION CURVE DOG #10



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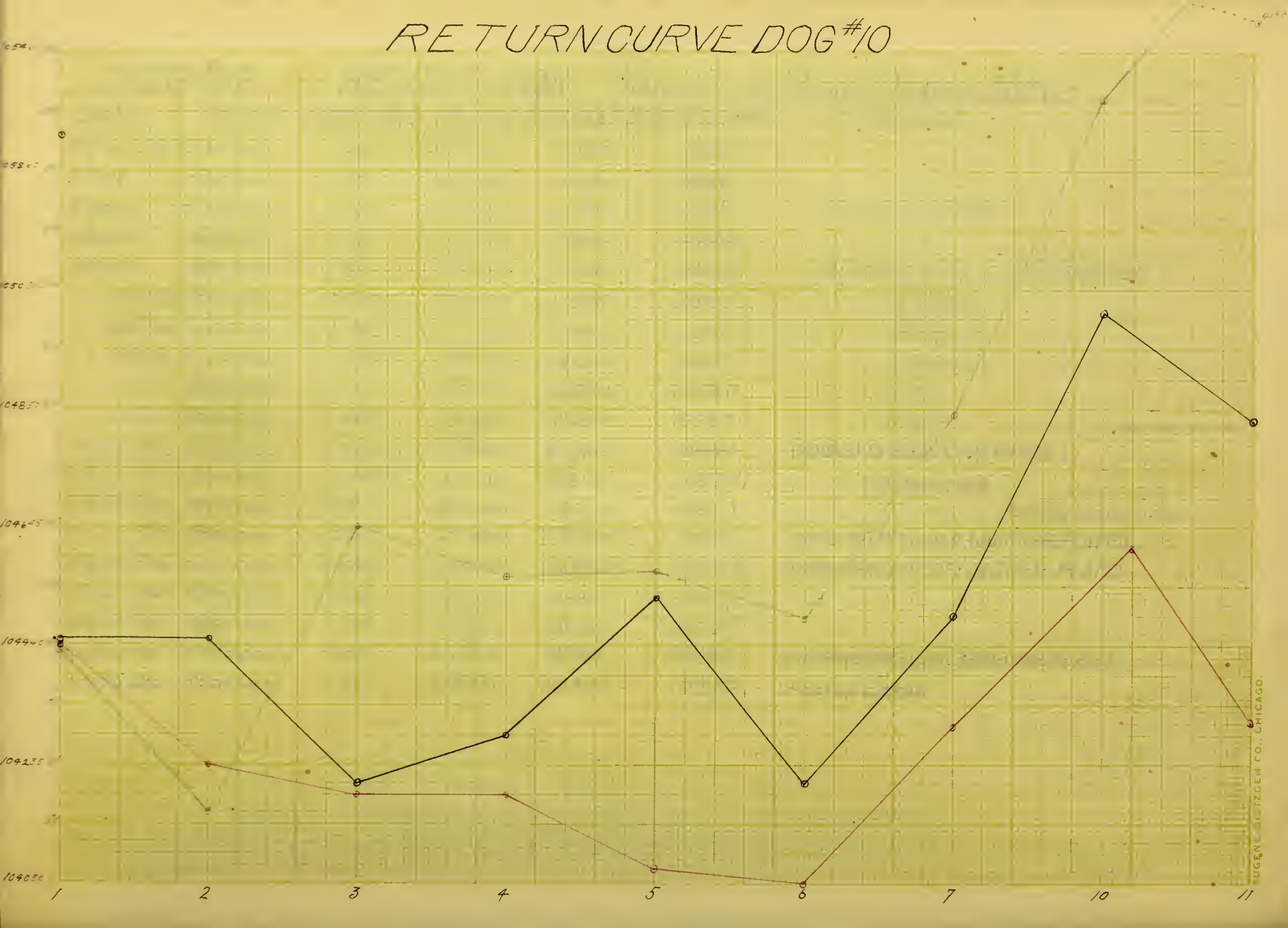
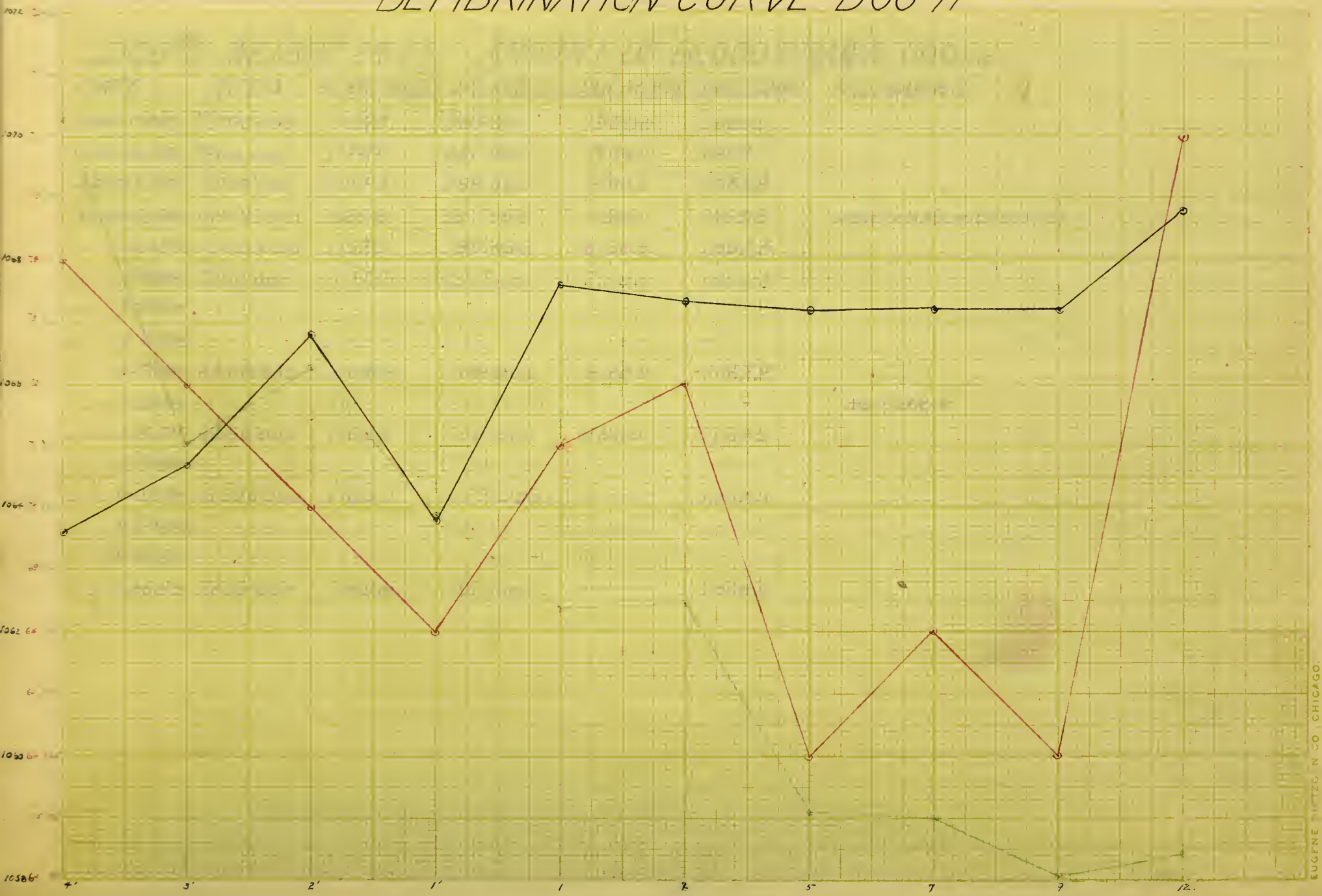


DOG #10 WEIGHT 11.9K AMOUNT OF BLOOD DRAWN 300 C.C.

DATE	REDS	PLATE:RED	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
NOV. 11 1902	5'000'000	1:14.1	357'000	21'000	1052.6	
NOV. 12, ..	5'000'000	1:14	357'000	10'800	1052.2	
NOV. 13,	5'000'000	1:16.6	300'000.	12'200	1052.3	PLATES LARGE
NOV. 14,	4'900'000.	1:15.3	320'000	9'400	1052.4	.. ..
NOV. 15,	5'800'000	1:99	575'000	11'300	1052.0	BEFORE FIRST DEFIBRINATION
.. .. 5:07 PM	5800'000	1:32.6	178'000	11'800	1052.0	.. .. SECOND .. .. ..
.. .. 3:30 PM	6'000'000	1:42.6	141'000	12'000	1052.2	.. .. THIRD .. .. ..
.. .. 3:52 PM	5'700'000	1:76.6	74'400	8200	1052.7	.. .. FOURTH .. .. ..
.. .. 4:3 P.M.	5900'000	1:112	42'700	12'000	1053.0	.. .. FIFTH .. .. ..
.. 4:59 P.M.	6200'000	1:278	22'300	12'000	1052.7	.. .. SIXTH .. .. .. ONLY TWO PLATES FOUND
NOV. 16 1902	4'000'000	1:40.2	99'500	20'400	1044.1	WOUND IN GOOD CONDITION
NOV. 17, 1902	3500'000	1:114(?)	30'700	21200	1044.1	.. .. SUPPURATING
NOV. 8, 1902	3400'000	1:22.7	150'000	13200	1041.7	.. .. PLATES USUAL SIZE
NOV. 19 1902	3400'000.	1:26.7	127'600	9'800.	1042.5	NOTHING PECULIAR ABOUT THE PLATES
NOV. 20, 1902	3'100'000	1:23.4	132200	10'800	1044.8	LARGE PERCENT OF LONG OVAL PLATES
NOV. 21, 1902	3'000'000	1:26.8	112'000	8'800	1041.7	.. ..
NOV. 22, 1902	3800'000	1:19.4	196'000	8800	1044.5	
NOV. 25, 1902	4'400'000	1:13.3	330'000.	8'200	1049.6	NOTHING PECULIAR ABOUT THE PLATES
NOV. 26, 1902	3800'000	1:9.1	418'000.	10'400	1047.8	PLATES LARGE

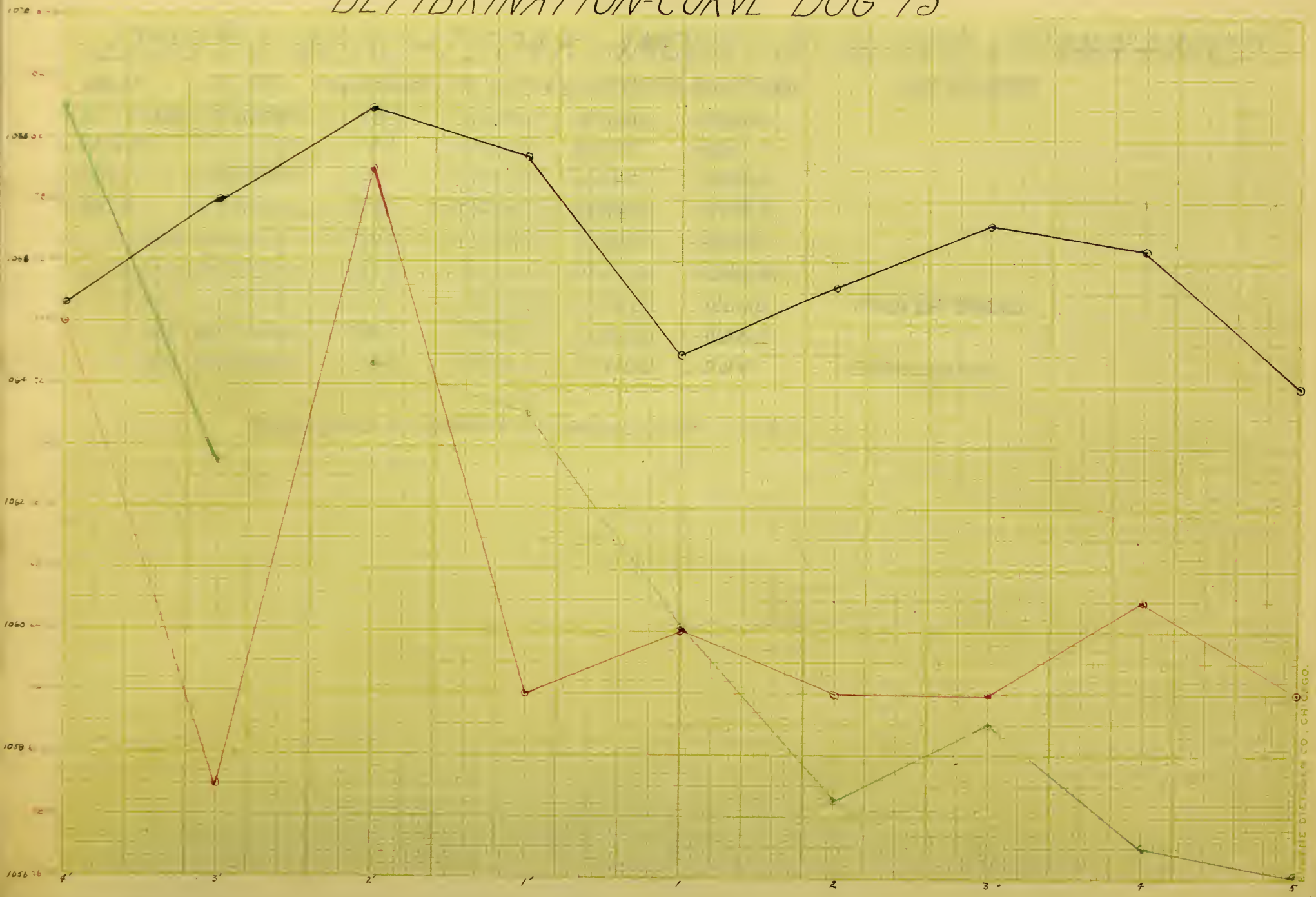
DEFIBRINATION CURVE DOG #11



DOG #11 WEIGHT 24.7K. AMOUNT OF BLOOD DRAWN 600 c.c.

DATE	REDS	PLATE:RED	PLATES	LEUCOCYTE	SPEC.GRAV.	REMARKS
NOV.17,1902	7'400000	1:19.3	383'000	9'000	1063.6	
NOV.18,1902	7'200 000	1:28.6	251'000	8'000	1064.7	
NOV.19,1902	7'000'000	1:24.3	288'000	8'000	1066.8	
NOV.20,1902	6800'000	1:26.2	221'000	7'900	1063.8	GREAT MANY MICROCYTES
" 3:43 P.M.	7'100'000	1:37.3	189'000	10'000	1067.6	
" 4:08 P.M.	7'200'000	1:37.2	193'000	7'000	1067.4	
" 4:29 P.M.	-------	------	------	-----	------	
" 4:40 P.M.	-------	-----	------	-----	-----	
" 4:48 P.M.	6600'000	1:64.4	102'000	6'200	1067.2	
" 5:02 P.M.	-------	-----	------	-----	-----	NO FIBRIN
" 5:14 P.M.	6800'000	1:67.7	100'000	7'800	1067.2	
" 5:50 P.M.	-------	-----	------	-----	-----	
" 6:02 P.M.	6'600'000	1:85.1	77'000	------	1067.2	
" 6:24 P.M.	-------	-----	------	------	-----	
" 6:35 P.M.	-------	------	------	------	------	
" 6:46 P.M.	7600'000	1:82.8	91'000	------	1068.8	

DEFIBRINATION-CURVE DOG #13

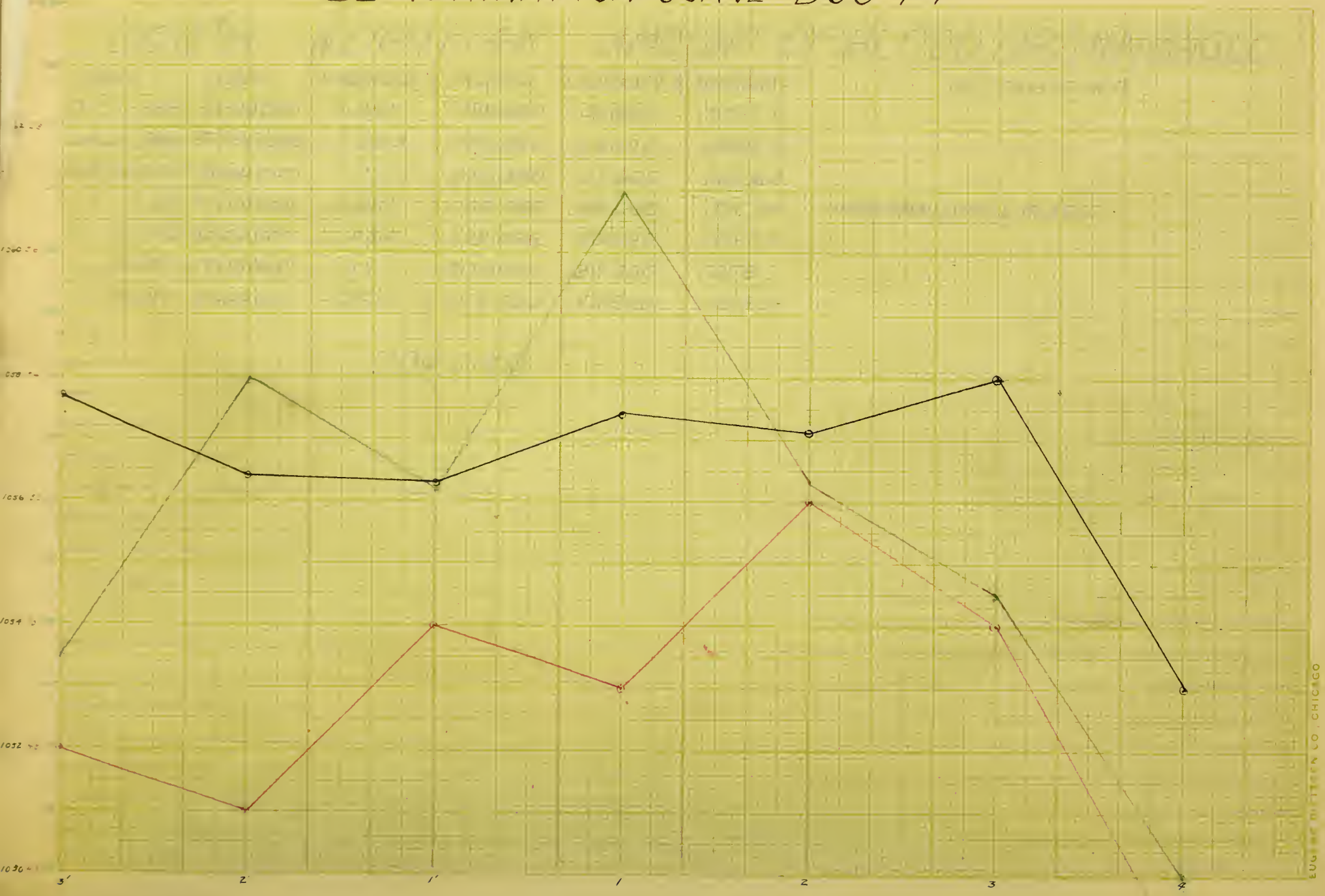


DOG #13 WEIGHT 20.4K AMOUNT OF BLOOD DRAWN 475 C.C.

DATE	REDS	PLATE:REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
DEC. 9 1902	7400000	1:10.2	725000	8000	1065.3	
DEC. 10	5700000	1:13.3	436000	9600	1067.	
DEC. 11	7900000	1:15.3	516000	13000	1068.5	
DEC 12	6200000	1:13	477000	13000	1067.7	
" " 1. DEF.	6400000	1:20.8	307000	12800	1064.5	
" " 2. DEF	6200000	1:37.3	166000	12400	1065.6	
" " 3 DEF	6200000	1:27.9	225000	11000	1066.6	PLATES SMALL
" " 4 DEF.	6500000	1:44.3	124000	10800	1066.2	
" " 5 DEF.	6200000	1:59.5	104.000	13000	1064.	FROM HEART

* DOG DIED OF HEART CLOT BEFORE LAST COUNT

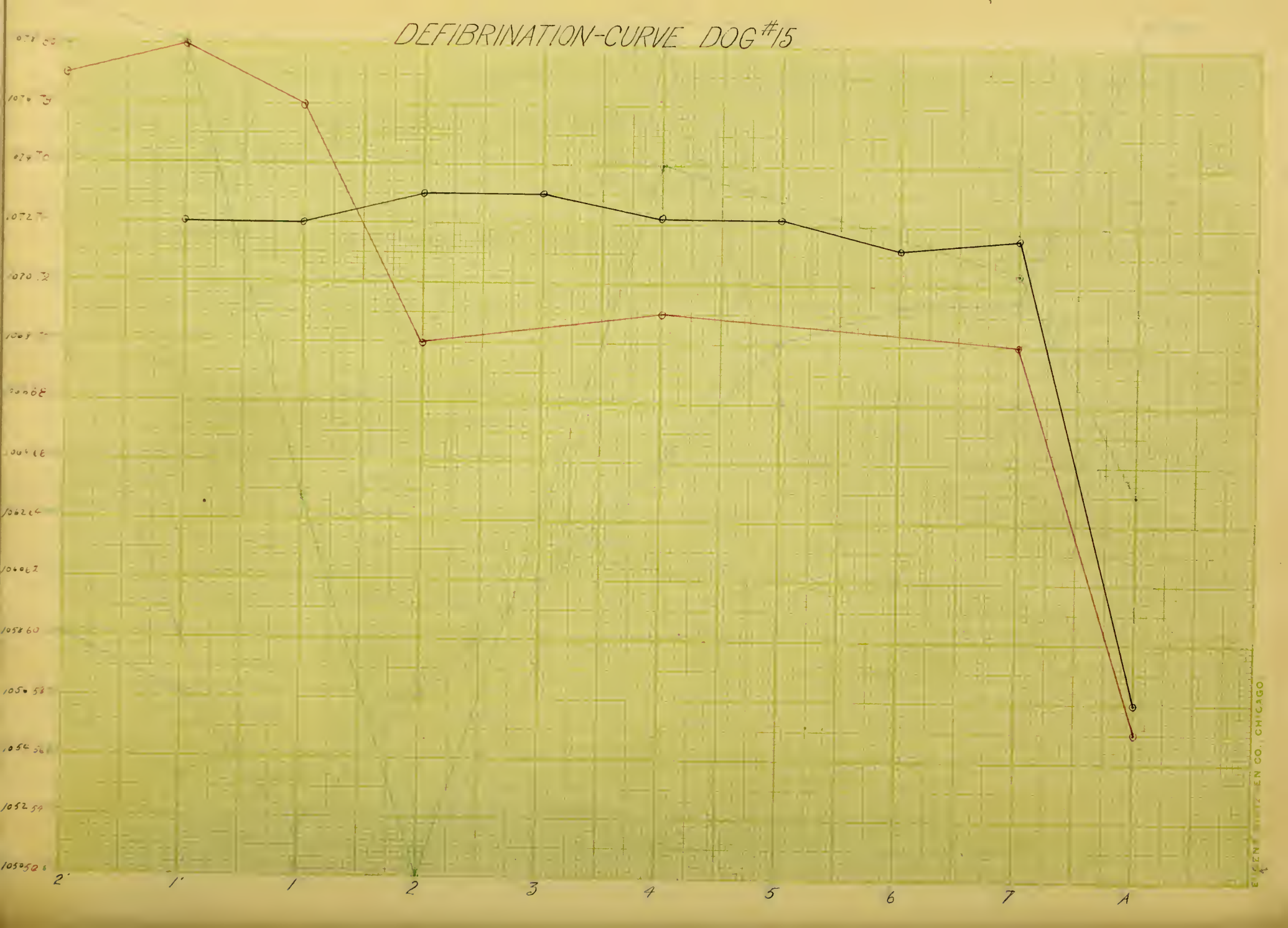
DEFIBRINATION-CURVE DOG #14

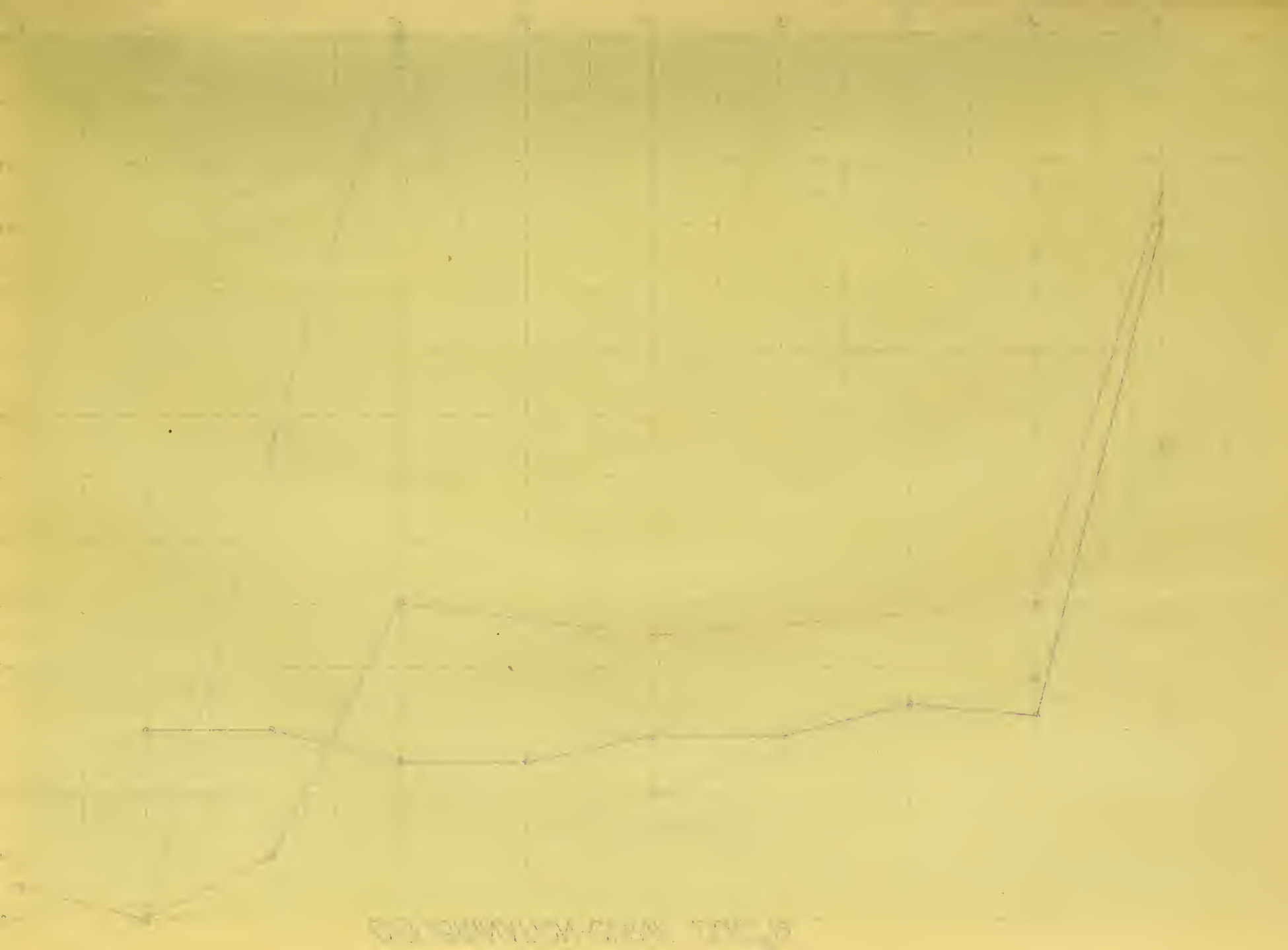


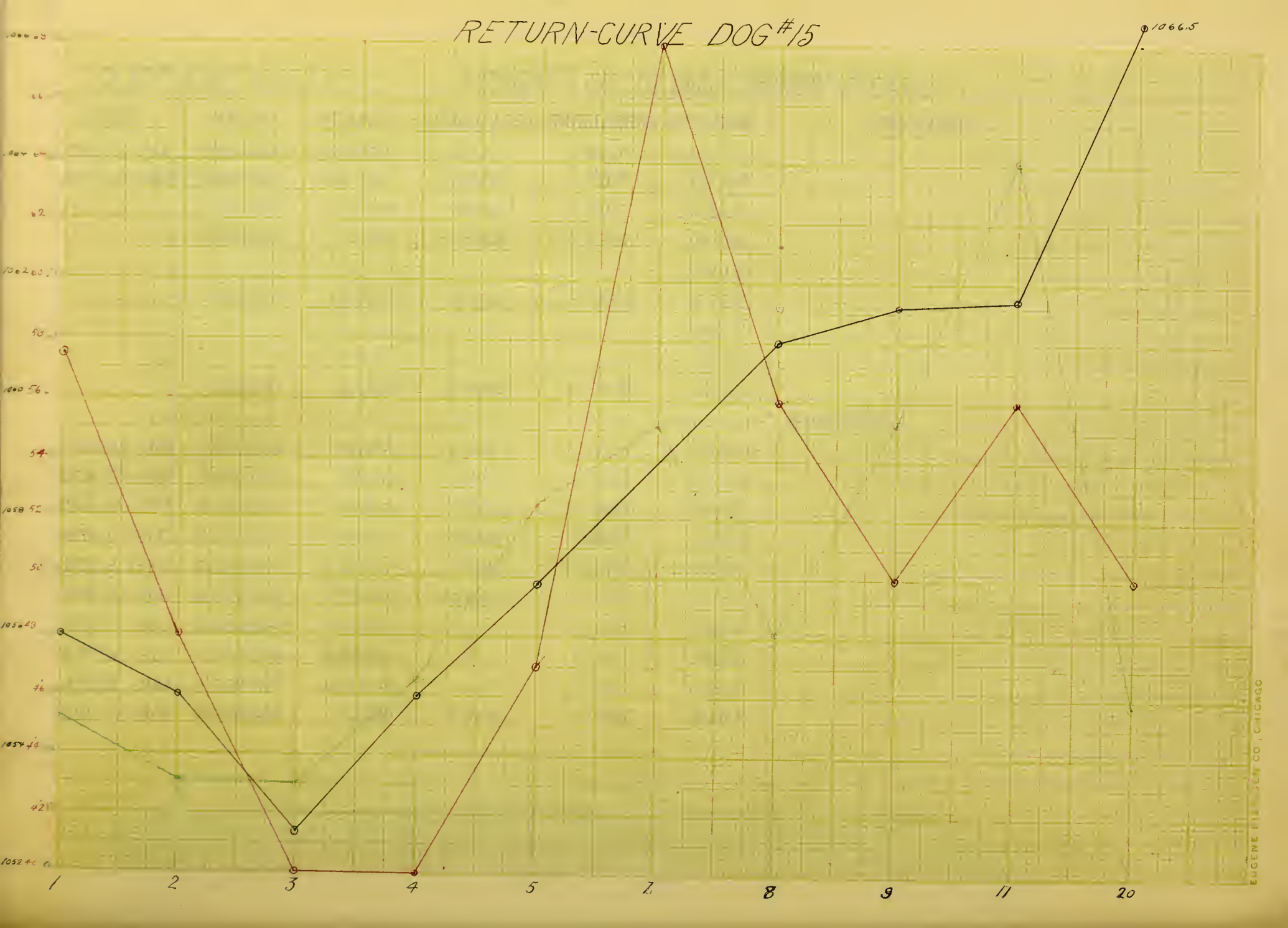
DOG #14 WEIGHT 14K. AMOUNT OF BLOOD DRAWN 350 C.C.

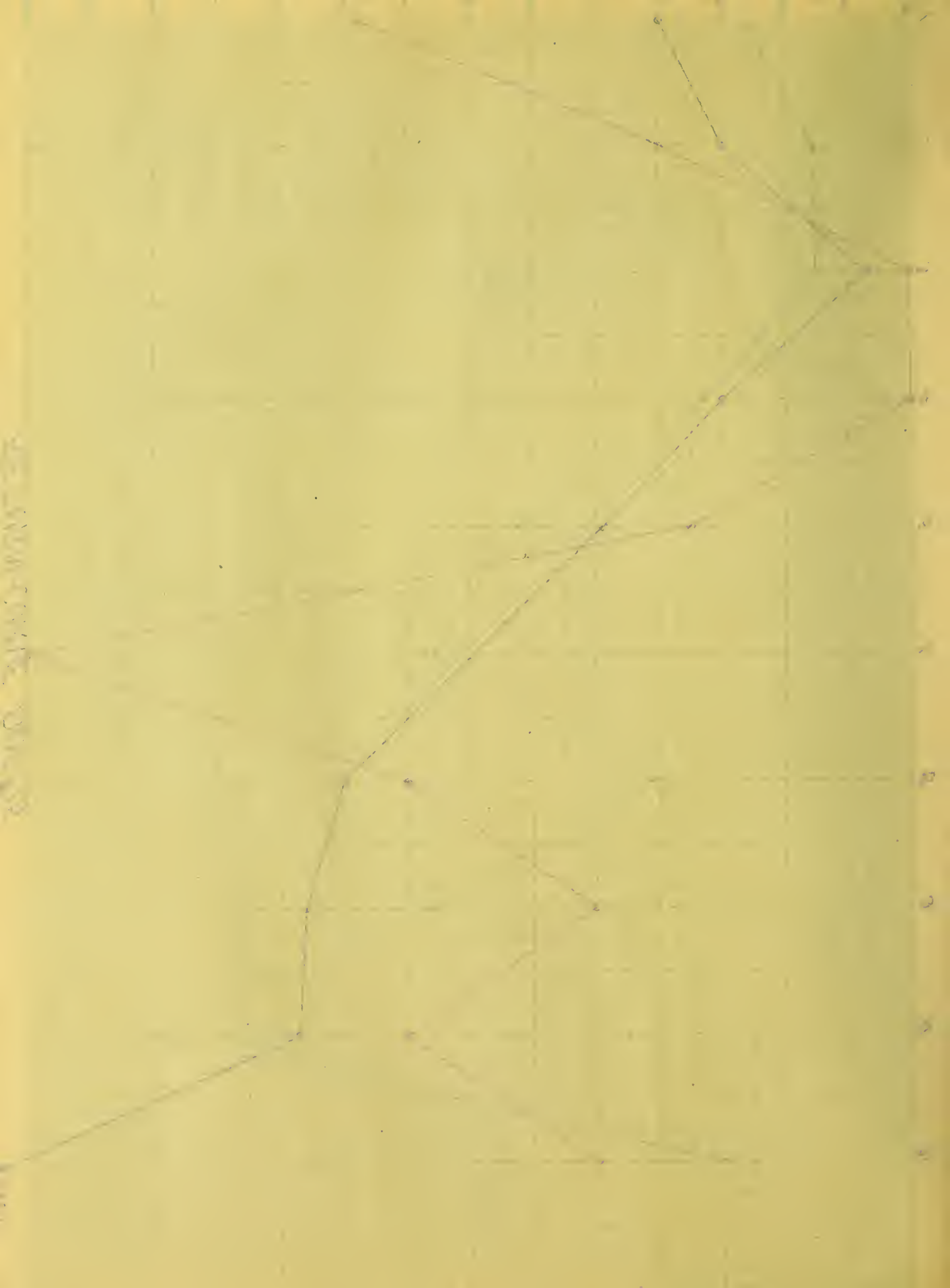
DATE	REDS	PLATE:RED	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
DEC. 14, 1902	4'800'000.	1:25.2	190'000	36'800	1057.7	
DEC. 16, 1902	4'700'000	1:20.3	234'000	26'300	1056.4	
DEC. 17, 1902	5000'000	1:22.9	218'000	27'600	1056.3	
" " 1 DEF.	4'900'000	1:18.5	264'000	26'000	1057.4	GREAT MANY SMALL PLATES
" " 2. DEF.	5200'000.	1:23.7	219'000	20'600	1057.1	
" " 3. DEF.	5'000'000	1:25.	200'000.	20'000	1058.	
" " 4 DEF.	4'000'000	1:25.7	155'000.	19'500	1053.4	

DOG DIED


DEFIBRINATION-CURVE DOG #15
2'
1'
1
2
3
4
5
6
7
A

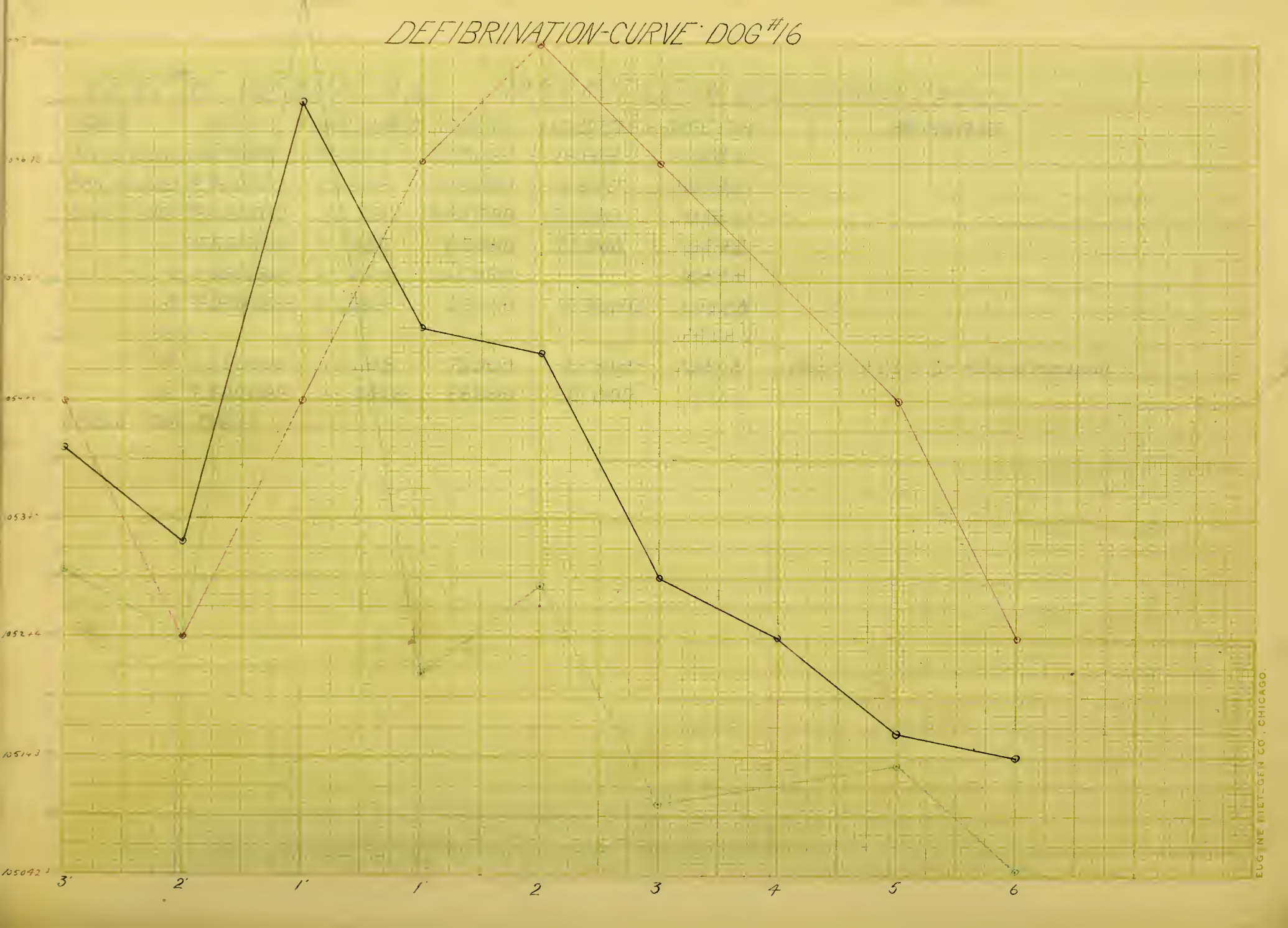


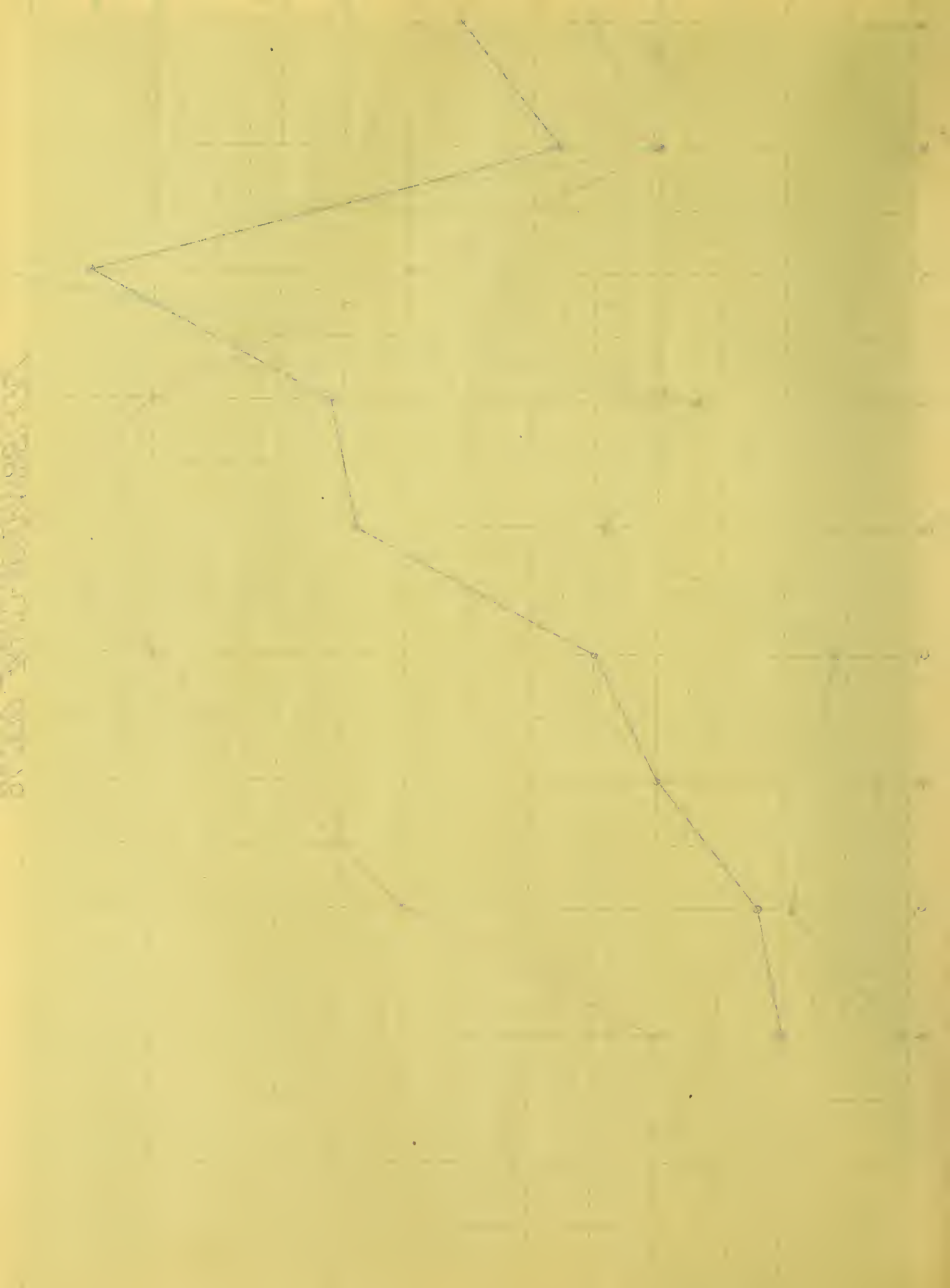

RETURN-CURVE DOG #15
1066.5
1
2
3
4
5
7
8
9
11
20



DOG # 5 WEIGHT 17.7 AMOUNT OF BLOOD DRAWN 425 c.c.

DATE	REDS.	PLATES	LEUCOCYTES	PLATES: REDS.	SPEC. GRAV.	REMARKS
MCH. 27, 1903	7'900'000	231'000	10'700	1 : 33.7	--------	
MCH. 28, 1903	8'000'000	206'000	6'800	1 : 38.8	1072.0	
" " " 1	7'800'000	129'000	7'800	1 : 60.5	1072.0	
" " " 2	7'000'000	65'000	11'000	1 : 107.3	1073.0	
" " " 3	---------	--------	--------	--------	1073.0	
" " " 4	7'100'000	185'000	8'000	1 : 38.3	1072.2	
" " " 5	---------	--------	--------	--------	1072.2	
" " " 6	---------	---------	--------	--------	1071.2	
" " " 7	7'000'000	167'000	10'200	1 : 418	1071.6	
" " " 8	---------	----------	---------	-------	----------	NO FIBRIN
MCH. 29, 1903	5'750'000	130'000	10'000	1 : 44	1056.0	
MCH. 30, 1903	4'800'000	79'000	16'000	1 : 60.6	1055.0	
MCH. 31, 1903	4'000'000	76'000	11'800	1 : 52.4	1052.7	
APR. 1, 1903	4'000'000	166'000	10'600	1 : 24.1	1055.0	
APR. 2, 1903	4'500'000	312'000	7'200	1 : 14.4	1056.9	
APR. 4, 1903	6'800'000	379'000	23'600	1 : 17.9	------	
APR. 5, 1903	5'600'000	202'000	--------	1 : 27.7	1061.0	
APR. 6, 1903	5'000'000	382'000	-------	1 : 13.1	1061.6	
APR. 8, 1903	5'600'000	602'000	------	1 : 9.3	1061.7	
APR. 17, 1903	5'000'000	145'000	5'900	1 : 34.5	1066.5	

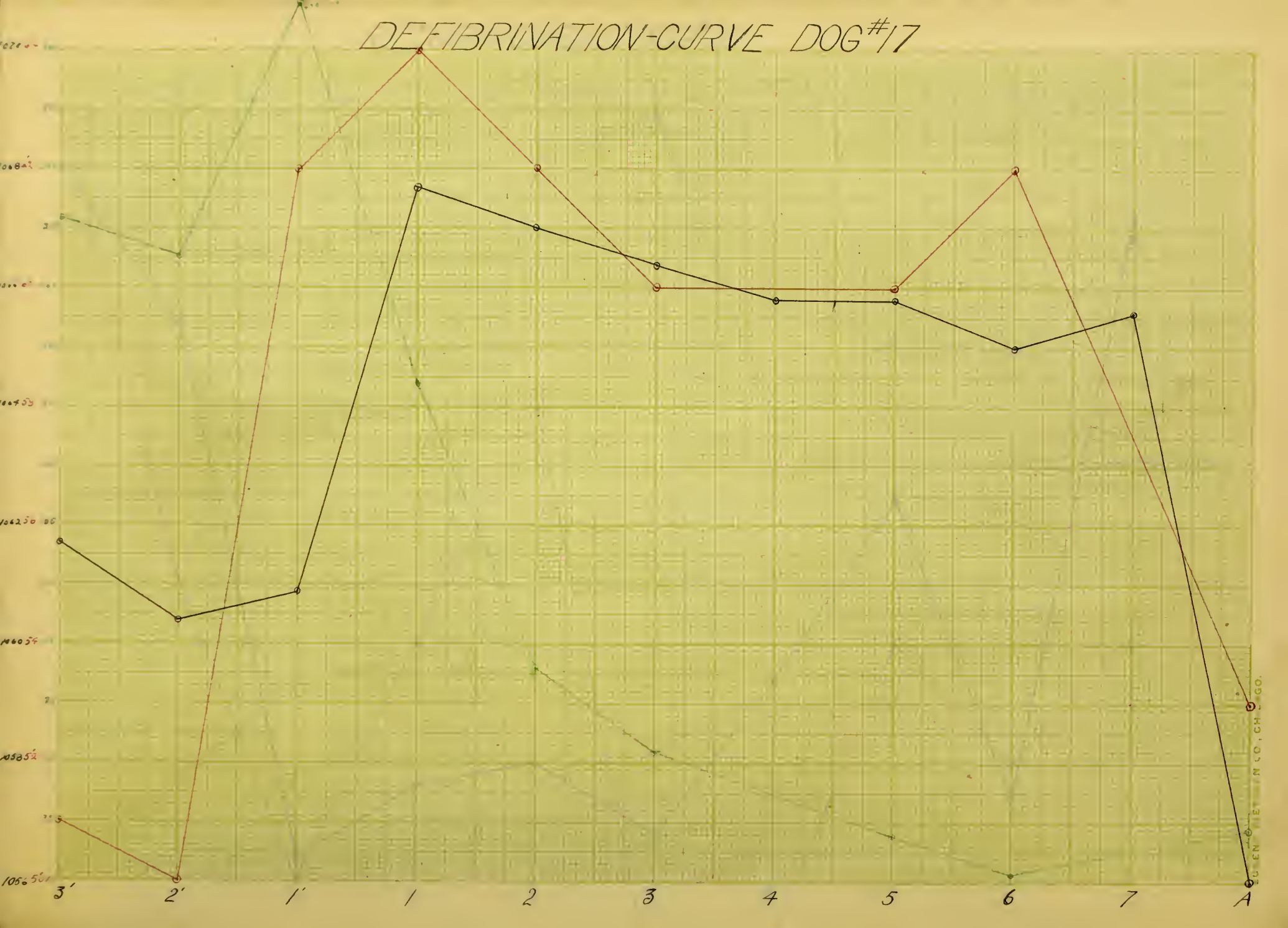

DEFIBRINATION-CURVE: DOG #16
3'
2'
1'
1
2
3
4
5
6
EUGENE FIET-GEN CO., CHICAGO.



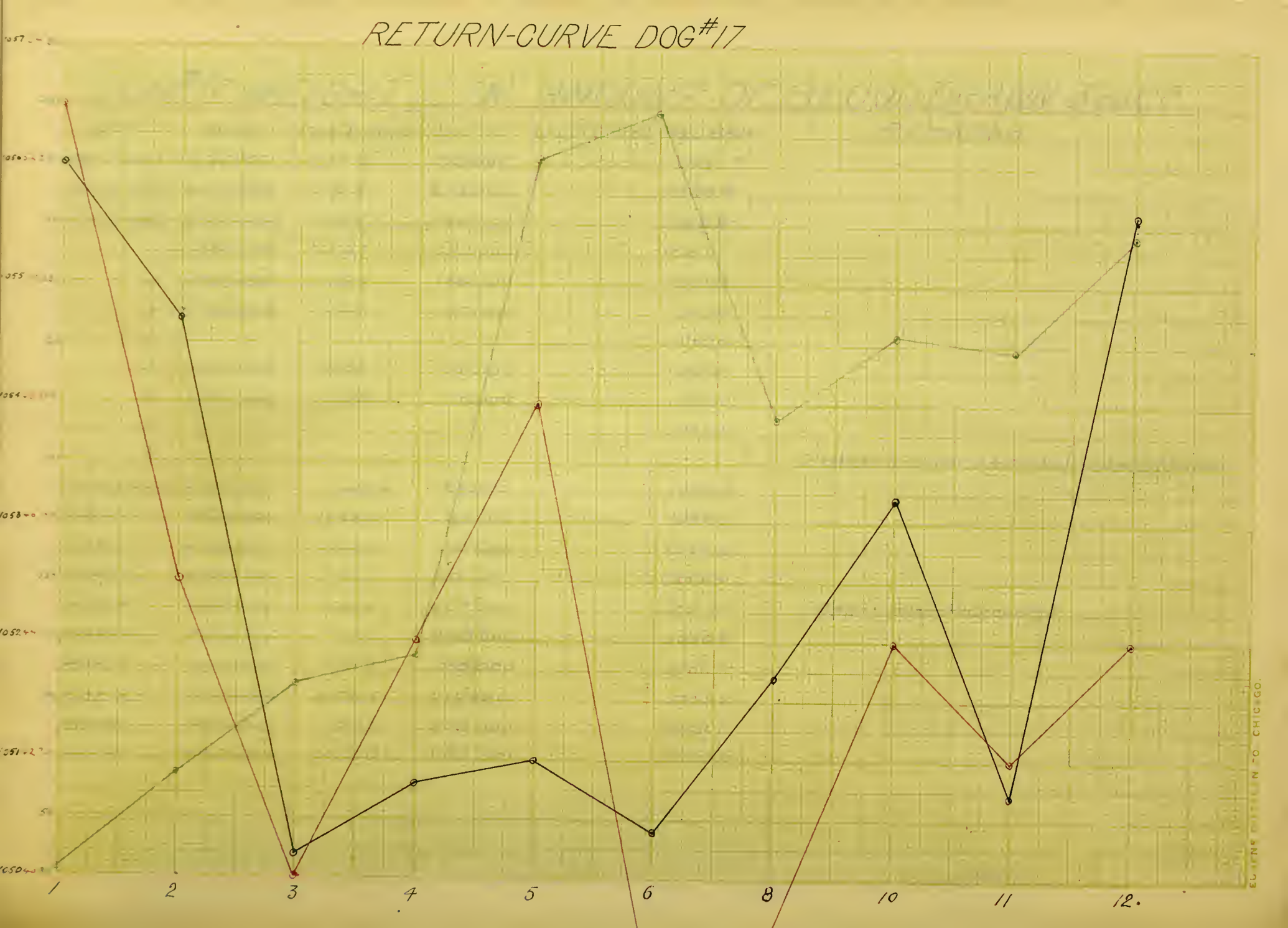
DOG #16 WEIGHT 9.K AMOUNT OF BLOOD DRAWN 225 C.C.

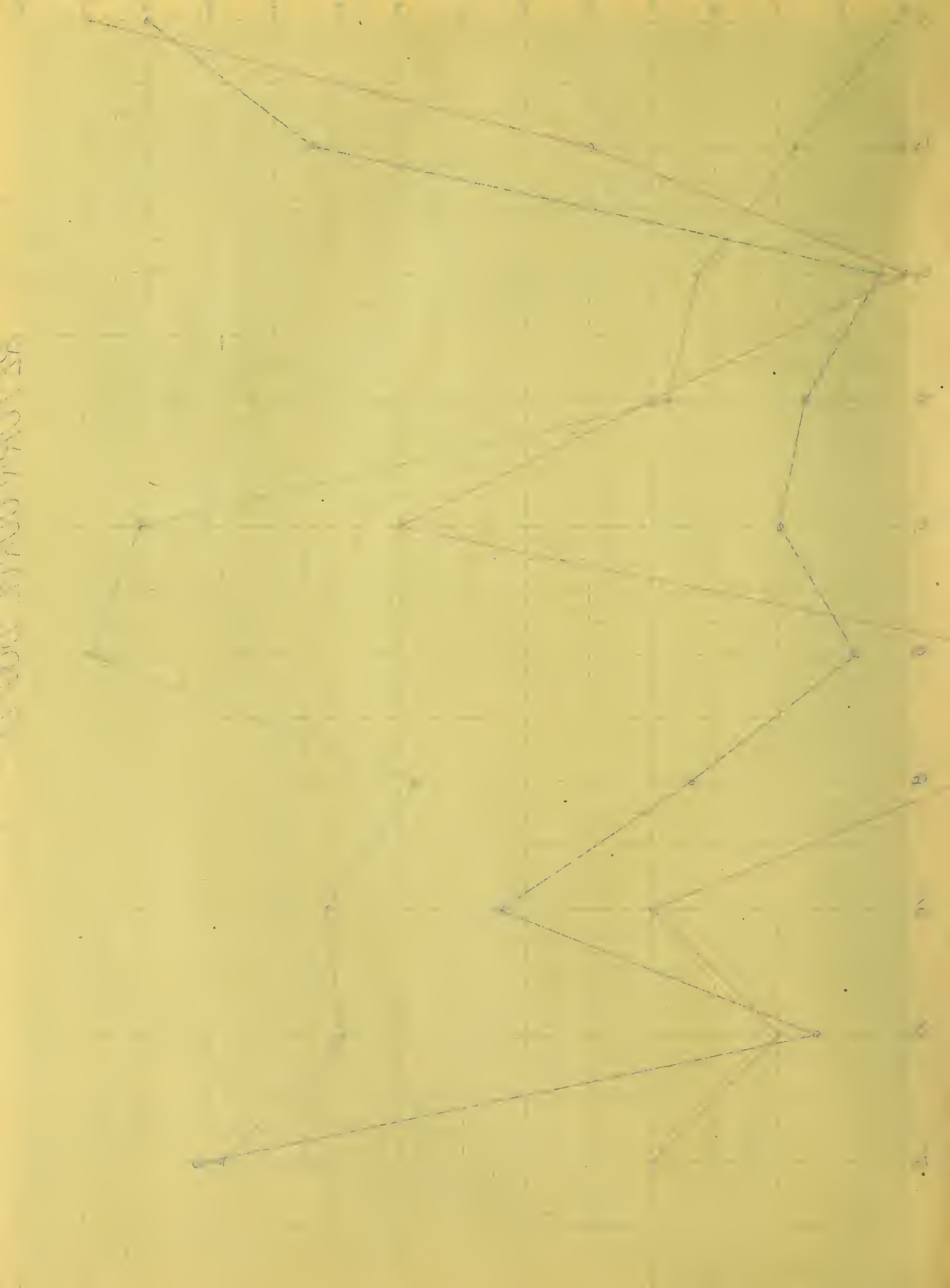
DATE	REDS	PLATES: REDS	PLATES	LEUCOCYES	SPEC. GRAV	REMARKS.
APR. 9, 1903	4'600'000	1:26.5	174'000	11'300	1053.6	
APR. 10, 1903	4'400'000	1:31.4	140'000	11'200	1052.8	
APR. 11, 1903	4'600'000	1:9.9	465'000	20'000	1056.5	
" " #1	4'800'000	1:386	122'000	22'900	1054.6	
" " #2	4'900'000	1:295	166'000	------	1054.4	
" " #3	4'800'000	1:86.4	56'000	11'900 (?)	1052.5	
" " #4	---------	------	-------	-------	1052.0	
" " #5	4'600'000	1:60.8	76'000	22'800	1051.2	ONLY 125 C.C OF BLOOD DRAWN
" " #6	4'400'000	1:2026	22'000	21'000	1051.0	

APR. 12 DOG DIED


DEFIBRINATION-CURVE DOG #17
3'
2'
1'
1
2
3
4
5
6
7
A

RETURN-CURVE DOG #17



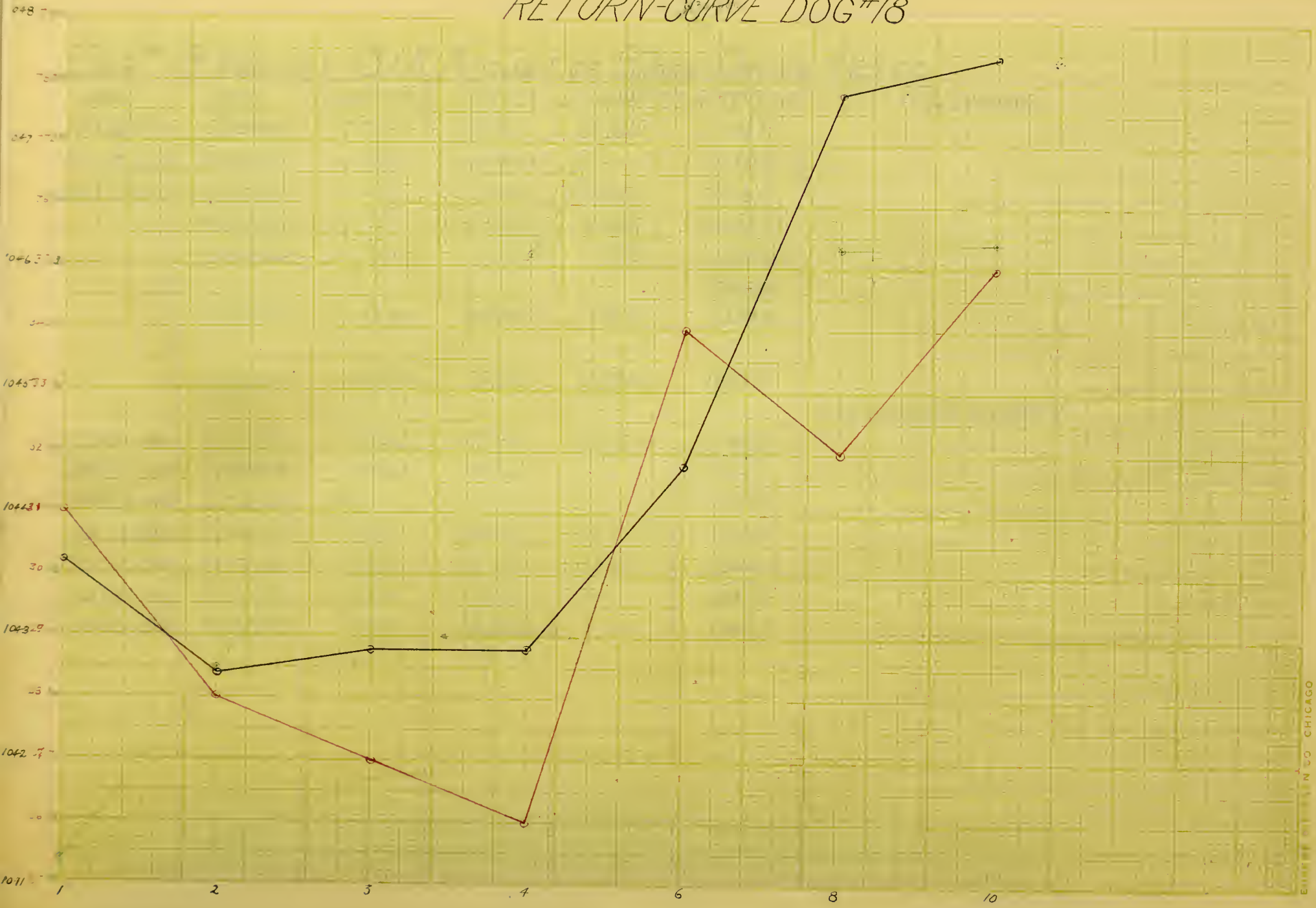


DOG #17 WEIGHT 12.7K AMOUNT OF BLOOD DRAWN 300 C.C.

DATE	REDS	PLATE:REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
MAY 7, 1903	5'100'000	1:17.6	289'000	.....	1061.7	
MAY 8, 1903	5'000'000	1:18.3	273'000	.....	1060.4	
MAY 9, 1903	6'200'000	1:15.5	400'000	.....	1069.9	
.. 1	6'400,000	1:29.1	220'000	.....	1067.7	
.. 2	6'200'000	1:62.2	100'000	.....	1067.0	
.. 3	6'000'000	1:92.5	65'000	.....	1066.4	
.. 4	.....	.....	.....	.....	1065.8	
.. 5	6'000'000	1:203	30'000	.....	1065.8	
.. 6	6'200'000	1:493	13'000	.....	1065.0	
.. 7	.....	.....	.....	.....	1065.6	
.. 8	.....	.....	.....	.....	.....	FIBRIN HAD NOT ENTIRELY DISAPPEARED
MAY 10. 1903	5'300'000	1:168.3	32'000	.....	1056.0	
MAY 11, ...	4'500'000	1:69.5	65'000	.....	1054.7	
MAY 12, ...	4'000'000	1:42.0	95'000	.....	1050.2	
MAY 13, ...	4'400'000	1:41.9	105'000	.....	1050.8	
MAY 14, ...	4'800'000	1:18.0	272'000	.....	1051.0	GREAT MANY MICROCYTES
MAY 15, ...	3'800'000	1:13.2	288'000	.....	1050.4	
MAY 17, ...	3'900'000	1:21.1	185'000	.....	1051.7	
MAY 19, ...	4'400'000	1:20.6	213'000	.....	1053.2	
MAY 20, ...	4'200'000	1:20.2	208'000	.....	1050.7	
MAY 21, ...	4'400'000	1:17.8	247'000	.....	1055.6	

DEFIBRINATION-CURVE DOG #18

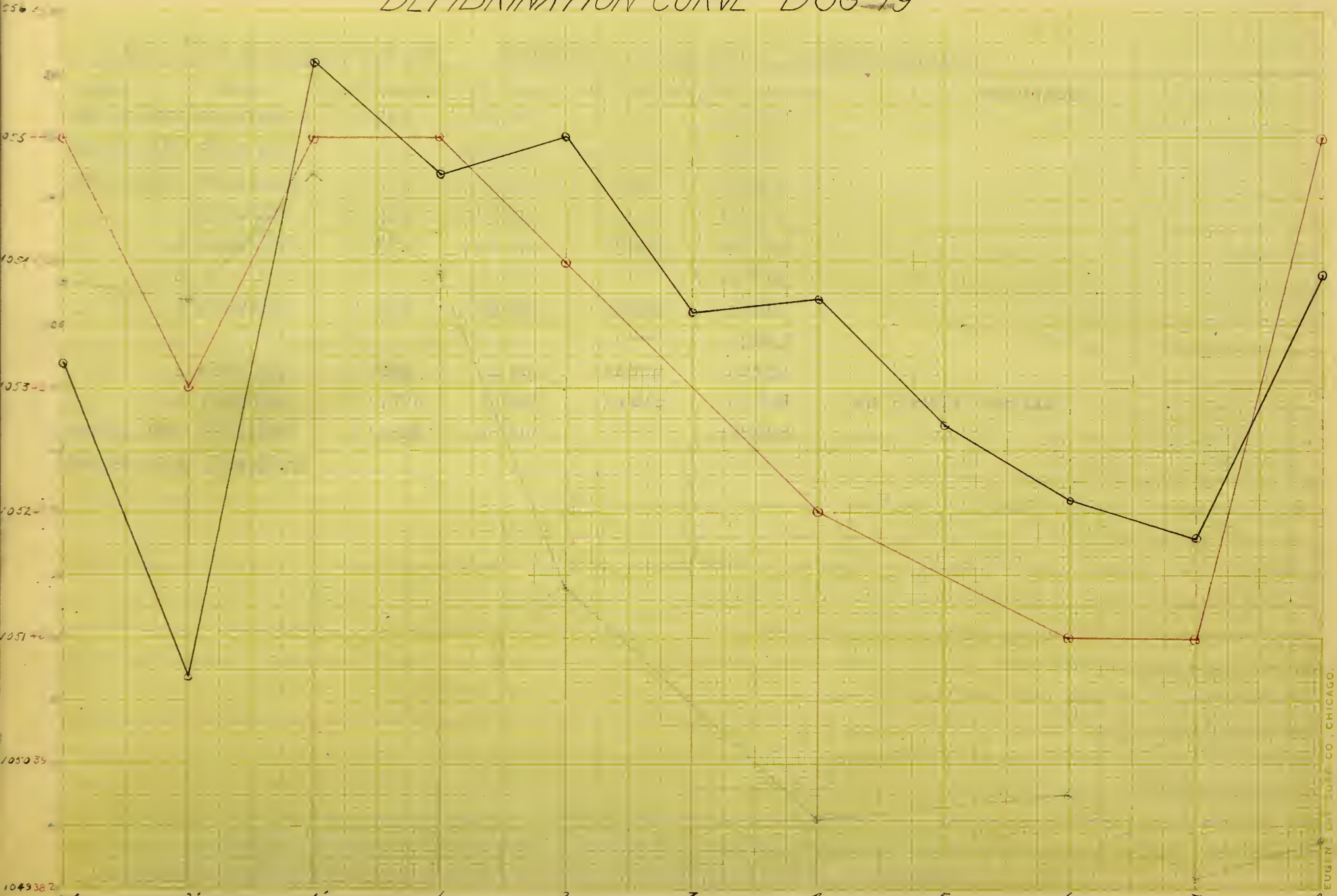
RETURN-CURVE DOG #18

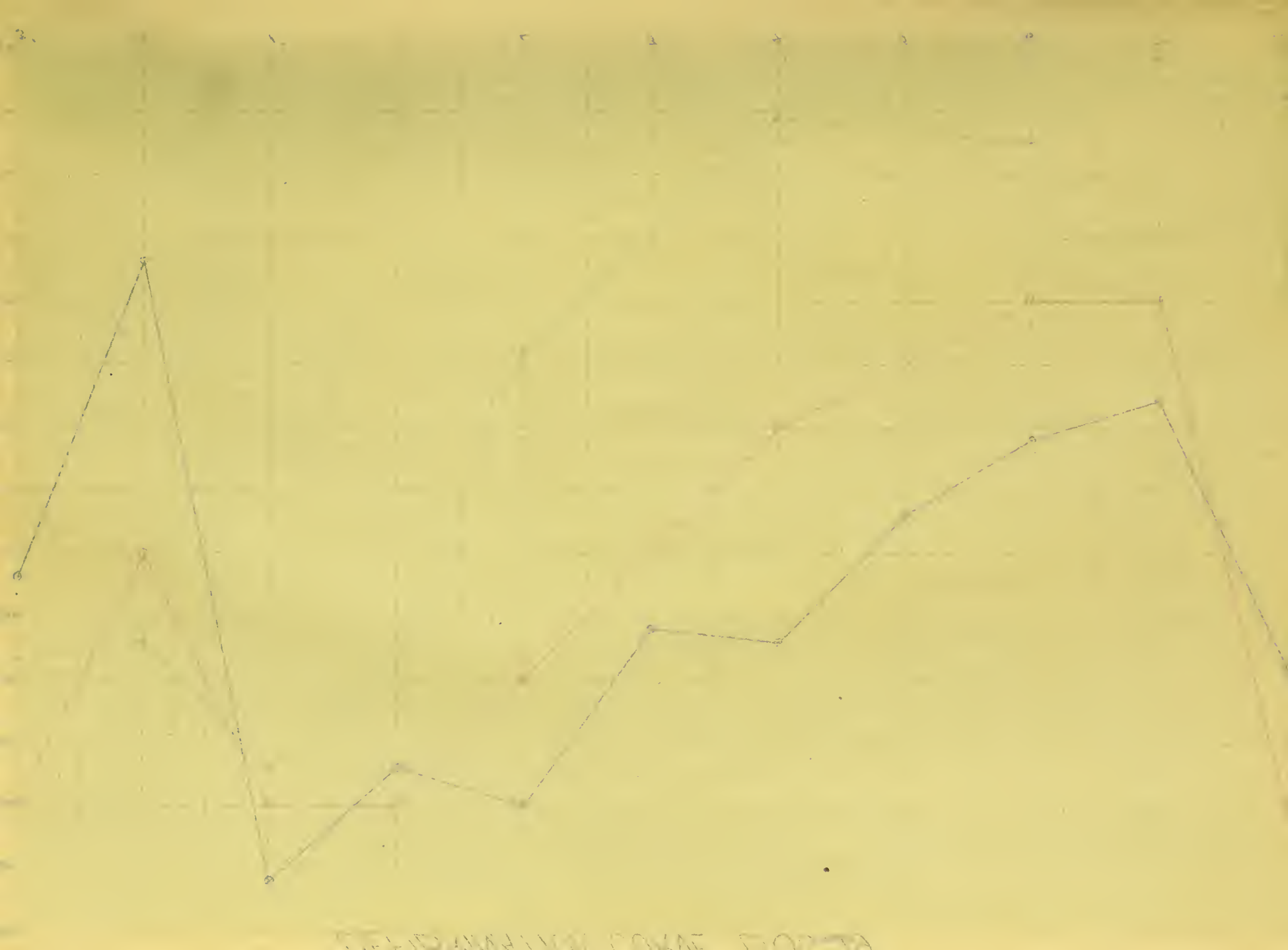


Dog #18 Weight 18.1K. Amount of Blood Drawn 425 c.c.

Date	Reds.	Plates: Reds	Plates	Leucocytes	Spec Grav.	Remarks.
May 3, 1903	5'700000	1:25.6	223000	25'000	1056.4	
May 4, 1903	4600000	1:28.2	163'000	-------	1053.8	
May 5, 1903	4'800'000	1:22.3	216'000	30'000	1056.2	
" " " 1	4'700000	1:77.5	61'000	11'400	1054.3	
" " " 2	4300000	1:75.9	58'000	7'700	1054.8	
" " " 3	----------	-------	-------	------	1054.0	
" " " 4	4000000	1:100.1	40'000	11'300	1053.2	
" " " 5	--------	-------	-------	--------	--------	
" " " 6	4'000'000	1:487.0	8'000	15'300	1051.5	
" " " 7	-------	-------	------	-------	-------	FIBRIN SIZE OF BEAN.
May 16, 1903	3'100'000	1:87.6	37'000	33'100	1043.6	
May 17, 1903	2'800'000	1:28.3	99'000	------	1042.7	
May 18, 1903	2'700'000	-------	----------	------	1042.9	
May 19, 1903	2'600'000	1:11.2	234'000	-------	1042.9	
May 21, 1903	3'400'000	1:10.6	321'000	-------	1044.4	
May 23, 1903	3'200'000	1:13.6	236'000	------	1047.4	
May 25, 1903	3'500'000	1:15.1	238'000	------	1047.7	

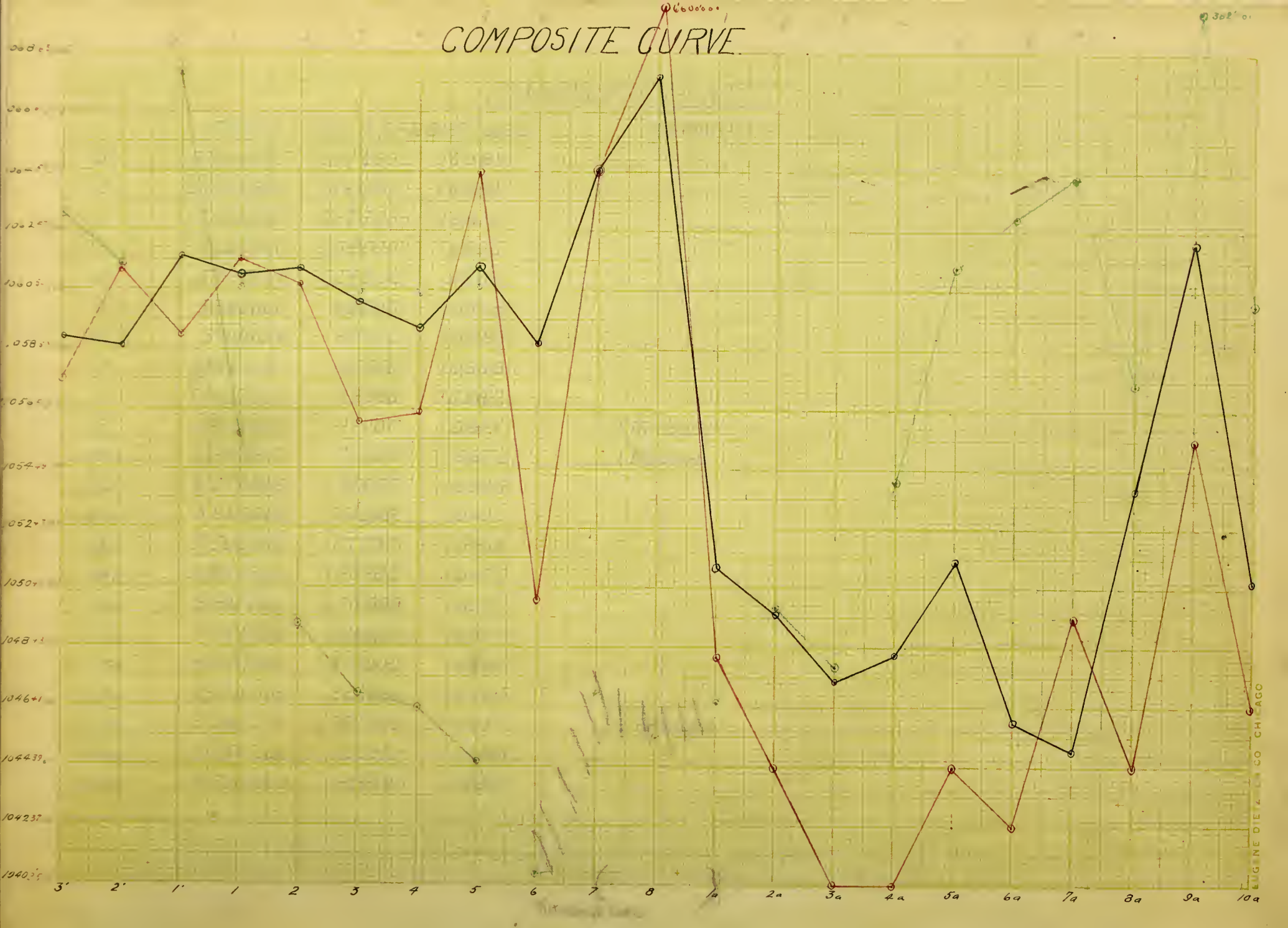
DEFIBRINATION-CURVE DOG #19





DOG #19 WEIGHT 12.7K AMOUNT OF BLOOD DRAWN. 300 C.C.

DATE	REDS	PLATES: REDS	PLATES	LEUCOCYTES	SPEC. GRAV.	REMARKS
MAY 19, 1903	4'400'000	1 : 20.6	214'000	----------	1053.2	
MAY 20, 1903	4'200'000	1 : 20.2	208'000	----------	1050.7	
MAY 21, 1903	4'400'000	1 : 17.8	248'000	24'700	1055.6	
" " " 1	4'400'000	1 : 20.4	216'000	----------	1054.7	
" " " 2	4'300'000	1 : 37.1	116'000	11'800	1055.0	
" " " 3	----------	-------	----------	-------	1053.6	
" " " 4	4'100'000	1 : 96.3	42'000	14'700	1053.7	
" " " 5	----------	------	----------	-------	1052.7	
" " " 6	4'000'000	1 : 80.6	50'000	12'900	1052.2	
" " " 7	4'000'000	1 : 171.0	23'000	16'000	1051.8	NO FIBRIN FORMED.
MAY 22, 1903	4'400'000	1 : 125.0	35'000	-------	1053.9	
MAY 23, 1903	DOG DIED.					


COMPOSITE CURVE
3'
2'
1'
1
2
3
4
5
6
7
8
1a
2a
3a
4a
5a
6a
7a
8a
9a
10a
EUGENE DIETZEN CO CHICAGO

COMPOSITE PLATE

	REDS	PLATES	SPEC. GRAV.	REMARKS
3'	5'200'000	297'000	1058.4	
2'	5'570'000	276'000	1058.1	
1'	5'370'000	357'000	1061.1	
1	5'600'000	204'000	1060.5	
2	5'520'000	124'000	1060.7	
3	5'060'000	96'000	1059.6	
4	5'090'000	89'000	1058.7	
5	5'900'000	67'000	1060.8	
6	4'460'000	19'000	1058.2	
7	5'900'000	97'000	1064.1	3 RECORDS
8	6'600'000	77'000	1067.2	1 RECORD
1a	4'270'000	92'000	1050.7	
2a	3'900'000	132'000	1049.1	
3a	3'500'000	107'000	1046.9	
4a	3'900'000	185'000	1047.8	
5a	3'900'000	275'000	1050.9	
6a	3'700'000	296'000	1045.5	
7a	4'400'000	313'000	1044.5	
8a	3'900'000	226'000	1053.3	
9a	5'000'000	382'000	1061.6	1 RECORD
10a	4'100'000	260'000	1050.2	
11a	4'500'000	409'000	1053.4	